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Page 37, paragraph 4, line 3, "therm" should be "them."
Front cover No. 3, line 3, under "Contents," "Russell L. LeBarron" should
be "Russell K. LeBarron."
Page 133, Literature Cited (6), publication date "1937" should be "1938."
Page 148, table 1, column 3, line 1, "1929" should be "1939."
Page 149, table 2, column 10 (Organic matter), ninth figure, ".68" should
be ".86."
Page 150, table 3, column 6, line 1, "March 6" should be "March 7."
Page 150, table 3, column 8, line 7, "69.8" should be "59.8."
Page 157, table 6, column 18 (19:1), line 1, "1.318" should be "0.318."
Page 158, table 7, column 5, line 9, "+.027" should be "+.027*."
Page 160, paragraph 3, line 3, comma after "interaction" should be
omitted.
Page 167, table 14, last column, fourth figure from bottom, "—.0126*"
should be "—.0226*."
Page 169, table 15, column 5, line 1, "—0.0012" should be "—0.0012+."
Page 173, table 17, column 4, box heading, "Average Ca" should be
"Average P."
Page 174, table 18, column 1, last line, "Mean" should be followed by
"of 16 replicated treatments in each locality."
Page 174, table 18, column 5, line 1, third figure ".0668" should be ".0068."
Page 179, table 21, second half of table, column 10 (Mean), "*Percent*"
should be "*Gm.*"
Page 181, table 23, column 7, line 11, "+8.26" should be "+8.26+."
Pages 249 and 250, figures 1 and 2 should be interchanged; legends are
correct as they stand.
Pages 251 and 263, figures 3 and 6 should be interchanged; legends are
correct as they stand.
Page 308, twelfth line from bottom, "species" should be "specimens."
Page 332, third line from bottom, "*p*-bromobenzene" should be "*p*-dibromo-
benzene."
Page 384, fourth line from bottom, "ever" should be "never."
Front cover of No. 12, line 3, under "Contents" and on page 427, author's
name "R. E. Lawson" should be "R. E. Larson."
Page 433, figure 5, "=" following MAJOR-FORM INDEX should be
deleted.
Page 433, eighth line from bottom, "x" should be "X."
Page 435, eighth line from bottom, "Volume-KD^2L" should be "Volume =
KD^2L."
Page 440, first line, "figure 9" should be "figure 8."

THE BIG VEIN DISEASE OF LETTUCE IN RELATION TO SOIL MOISTURE [1]

By Dean E. Pryor [2]

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INTRODUCTION

Two diseases of unknown cause, big vein and brown blight, have been closely associated with the culture of lettuce (*Lactuca sativa* L.) in parts of California and Arizona since about 1917. Big vein has also been reported in certain places along the Atlantic seaboard. [3] The development of strains resistant to brown blight, [4] with the subsequent elimination of this disease from most commercial lettuce fields, has brought increased prominence to the less serious but as yet uncontrolled big vein. The only detailed published investigation of the disease was made by Jagger and Chandler. [5] According to their results the amount of disease was not reduced by soil applications of various major and minor elements or by prolonged leaching with water, but partial sterilization with steam or formaldehyde eliminated the causal agent. They also found that the disease could not be readily transmitted except through the soil and that it appeared to be influenced considerably by environment.

From the available data on big vein three lines of research, each contributing toward a solution of the problem, seem logical. These are (1) a determination of the identity of the causal agent and its relation to the plant, (2) a study of the environmental factors affecting the development of the disease, and (3) a search for resistant strains of lettuce. Although knowledge of the causal agent is desirable, at present it does not seem absolutely essential since another obscure disease, brown blight, has been controlled through the use of resistant varieties though its cause is not known. [6] Inasmuch as transmission has been obtained only through infective soil, and temperature and soil moisture have been suggested as important in the production of symptoms, [7] the influence of environment must be understood before satisfactory resistance trials can be instituted. As one phase of this investigation a study was made of soil moisture in relation to big vein. The results are reported in the present paper.

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MATERIALS AND METHODS

Lots of infested soil from lettuce-growing districts near El Centro in the Imperial Valley of California and near Salinas, Calif., were employed in all the experiments reported herein. The experiments were conducted at Torrey Pines near La Jolla, Calif. Both soils are of fine texture and have a moisture-holding capacity of about 50 percent.[8] One-gallon glazed crocks were filled with equal amounts of soil, and water was added through a central ⅜-inch pipe, slotted at ½-inch intervals, to bring the moisture to 35, 45, 55, 70, and 85 percent of the moisture-holding capacity in the different series. The pots were frequently brought up to weight, but when transpiration was unusually high, it was sometimes necessary to carry the two lower moisture levels slightly overweight so that the plants in these series would remain alive. When it was desired to grow successive crops of lettuce on the same lot of soil, the soil was removed from the pots, mixed thoroughly, and fertilized, the moisture-holding capacity was redetermined, and the soil was replaced in the pots and brought to the desired moisture level before replanting it.

It is recognized that these methods have certain very definite limitations. For example, Hendrickson and Veihmeyer [9] have shown that adding a small amount of water to the top of soil in a container saturates the upper part to field capacity, while the lower part remains in its original dry state for long periods. Without rather elaborate equipment, which was not entirely suited to the present work, it was not possible to maintain a moisture-holding capacity of a given percentage throughout the unit volume of soil used. However, the amount of water supplied over the growing period employed was regulated with some degree of accuracy even though moisture distribution in the soil was not so uniform as might be desired. Had the amount of water in the soil that was held at 35 percent of the moisture-holding capacity been evenly distributed, it is likely that sufficient moisture would not have been present for plant growth. Also, if the moisture-holding capacity had been determined on undisturbed field soil, the figure undoubtedly would have been different from the one given. These facts should be kept in mind when considering the effect of moisture on big vein in the present work.

Two noncommercial strains of lettuce were used because of their resistance to downy mildew, which would have interfered with studies on susceptible strains. Since the two strains do not differ in susceptibility to big vein, they are treated as one variety in the data reported. Seed was sown in soil from areas where big vein does not occur, and when one or two true leaves had unfolded on the plants, four plants were transplanted to each pot. The pots were placed on an outdoor bench which could be sheltered when there was danger of rain. In each experiment the pots for the different moisture levels were randomized in 10 blocks so that variance analyses [10] of the results could be made.

[8] RIKER, A. J., and RIKER, R. S. INTRODUCTION TO RESEARCH ON PLANT DISEASES. A GUIDE . . . 117 pp., illus. St. Louis, Mo. 1936.

[9] HENDRICKSON, A. H., and VEIHMEYER, F. J. MOISTURE IN SOIL IN CONTAINERS. Plant Physiology 16: 821–826, illus. 1941.

[10] SNEDECOR, GEORGE W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 3, 422 pp., illus. Ames, Iowa. 1940.

As big vein appeared on the plants, the date and the number of leaves affected were recorded. Experiments were discontinued after 2 to 3 months when the plants at higher moisture levels were becoming crowded. Crowding usually became noticeable just before the plants began to head. At the termination of the experiments, the tops were weighed to the nearest 0.1 gm. In some experiments the top weights were tabulated in the order in which the plants in each of the crocks became diseased, so that the relation between time of symptom appearance and final weight could be determined. This procedure was followed to learn whether the disease reduces weight perceptibly during a period roughly about half of the growing season under field conditions.

EXPERIMENTAL RESULTS

EFFECT OF SOIL MOISTURE ON SYMPTOM PRODUCTION

Symptoms of big vein were complicated in a few instances by the appearance of small, mottled plants, the leaves of which were often deformed and tended to curl downward at the margins. This type of abnormality, termed "rosette," was often found on plants growing in soil where big vein occurred, but sometimes it was found on plants growing in soil where this disease did not occur. In the writer's experiments these rosette plants were not considered as having big vein unless they showed the typical vein clearing and banding.

Big vein in some representative leaves is shown in figure 1, *A*. The plants in the lower moisture series, particularly those at 35 percent of the moisture-holding capacity, were generally darker green than those in the higher moisture groups, and the symptoms often were not distinct because vein clearing and lack of chlorophyll along the veins were less pronounced than in plants supplied with more water. Plants in the four-leaf stage or older and leaves of almost any age became diseased in these experiments, but generally the most typical symptoms were found on leaves of intermediate age. In the oldest leaves big vein was often very faint or absent, and in the youngest leaves, which were still somewhat curled, the disease was seldom evident. From the leaf on which initial symptoms appeared, the disease usually extended more rapidly to the younger leaves than to the older. Vein clearing and banding often varied in severity from time to time, apparently being influenced by environmental conditions other than soil moisture. Temperature is undoubtdly very important.[11]

The statistical significance of differences between adjacent moisture percentages changed from experiment to experiment and with the various lots of soil employed. For example, in one experiment with Salinas soil the number of big vein plants occurring at 35 percent of moisture-holding capacity was significantly less than at any other moisture level. In another experiment with the same soil, the disease incidence at 35 percent was significantly less than that at 55, 70, or 85 percent, while the 45-percent group produced significantly fewer diseased plants than the 70- or 85-percent series. Considering this variation, it seemed more important from a practical standpoint to establish the over-all effect of moisture on disease incidence than to

[11] Thompson, R. C., and Doolittle, S. P. See footnote 3, p. 1.
Jagger, I. C., and Chandler, N. See footnote 5, p. 1.
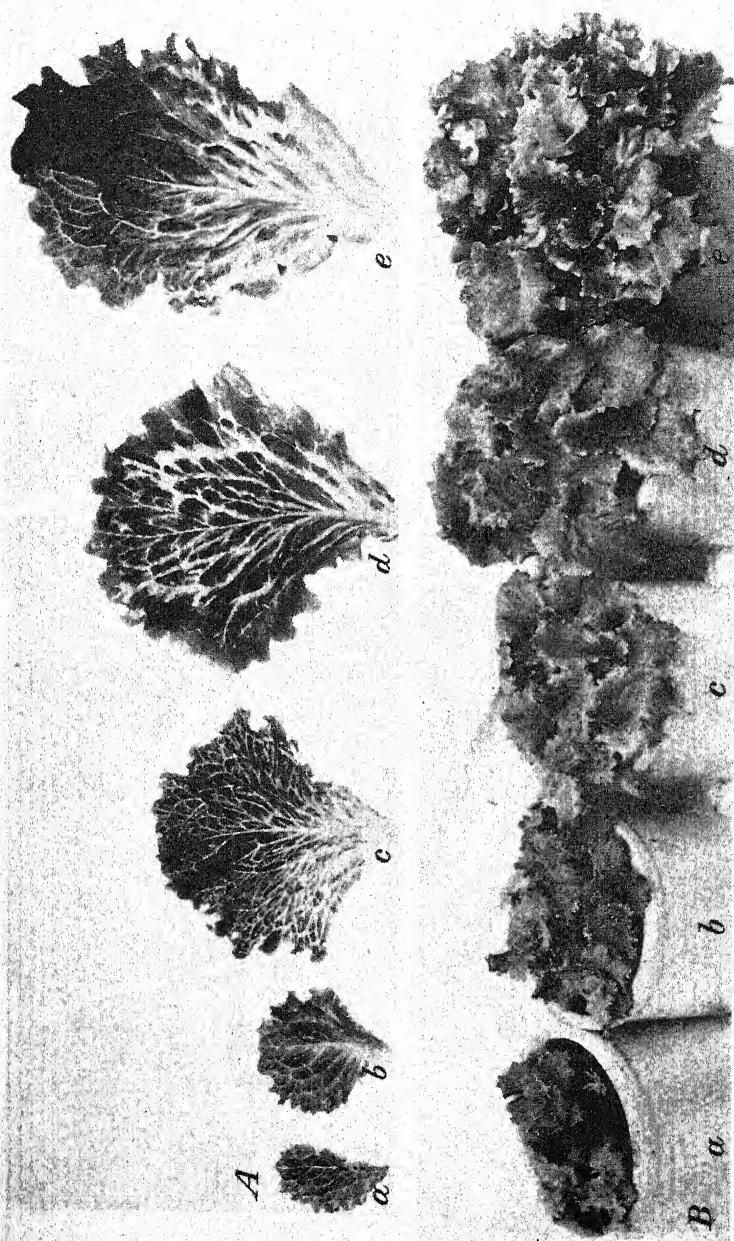
FIGURE 1.—*A*, Big vein symptoms on representative leaves from plants grown on soil of different moisture levels: *a*, 35 percent of moisture-holding capacity; *b*, 45 percent; *c*, 55 percent; *d*, 70 percent; and *e*, 85 percent. The leaves in *a*, *b*, and *c* show the usual type of vein clearing and banding and those in *d* and *e* show a more extensive chlorophyll deficiency along larger veins, a symptom of rare occurrence. *B*, Comparative sizes of plants grown at the respective moisture levels explained in *A*.

determine differences between individual levels, which in these experiments were composed of rather small moisture increments. With this in mind the results from several experiments have been recorded graphically and are shown in figure 2.[12]

An examination of figure 2 shows that soil moisture profoundly affected the number of big vein plants, the percentage tending to increase with an increase in the supply of water. There was a wide divergence between the two soils at moisture levels of 45, 55, and 70 percent of the moisture-holding capacity, the Imperial Valley soil having more diseased plants at these levels than the Salinas soil.
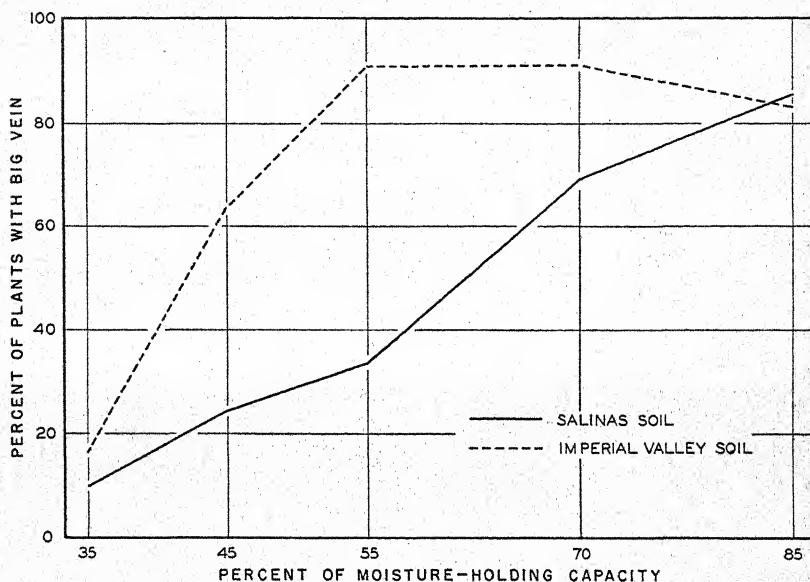
FIGURE 2.—The percentage of plants with big vein occurring on soils from Salinas and the Imperial Valley, held at different levels of moisture. Each point on the curve for the Salinas soil was based on 120 plants; for the Imperial Valley soil, on 79 to 90 plants.

This discrepancy was probably due largely to the difference in rate of growth but also perhaps to inherent differences between the soils. In general lettuce grows as well in the Imperial Valley as in the Salinas area. The former soil, however, has a rather high salt content and, when it was used in pots without drainage as in these experiments, the salinity built up to a point where the plants did not develop so rapidly as they did in the Salinas soil. Other explanations of the decreased fertility under conditions of poor drainage might be made, but at present this one seems the most logical. At any rate, since the plants in Imperial Valley soil grew more slowly, it was possible to continue the moisture treatments for a longer period than was feasible with the plants in the Salinas soil.

[12] Part of the data for the Imperial Valley soil were taken from the notes of the late I. C. Jagger.

Figure 3 indicates that big vein developed at about the same rate in both soils. However, almost all the big vein appearing on the plants after about 50 days from transplanting was in the series at 45 and 55 percent of the moisture-holding capacity. From this observation it appears that, if it had been possible to prolong the trials with Salinas soil, it is probable that the discrepancy between the two curves in figure 2 would have been reduced considerably.

The data of the foregoing discussion were based on plants grown from the one- or two-leaf stage to just before the heads began to form. Heading or seeding plants might conceivably present a somewhat different picture because their response to the environment is probably not the same as that of younger plants. Since field observations
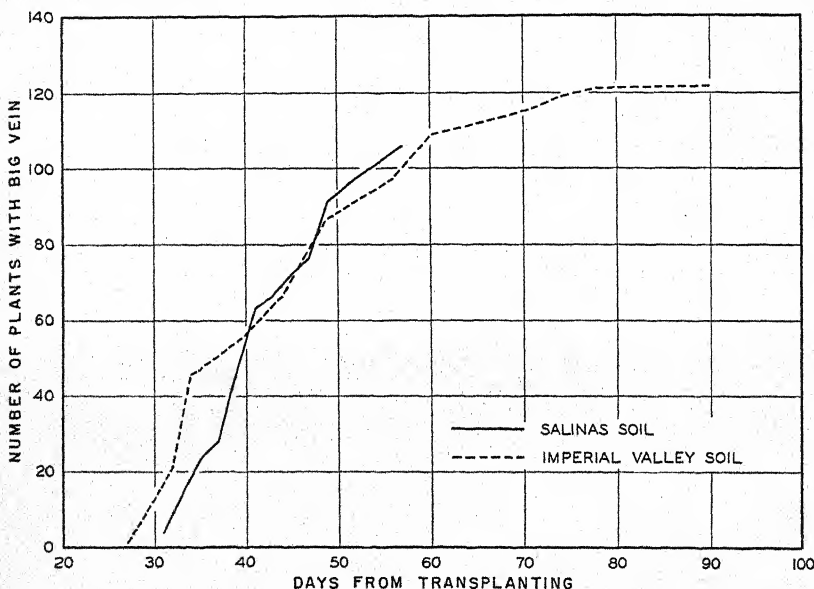
FIGURE 3.—Number of diseased plants in relation to number of days after transplanting as shown by a representative experiment in which 200 plants were grown on soil from Salinas and a similar experiment in which 200 plants were grown on soil from the Imperial Valley.

indicate that big vein is more likely to cause severe damage when plants are affected while young, a study of this early stage is of the most importance.

EFFECT OF SOIL MOISTURE AND BIG VEIN ON GROWTH

In table 1 are expressed the growth results from one representative experiment on each of the two soils studied. Figure 1, *B*, shows the comparative size of such plants. As would be expected, the different amounts of soil moisture had considerable effect on plant weight per pot. In all the experiments the largest yield was always obtained at the highest moisture level, although this level was probably somewhat above the optimum for field-grown lettuce. Since no allowance was

made for increase in plant weight as the experiment progressed, 85 percent of the moisture-holding capacity was above the optimum when the trial was started; but, as the plants grew, correspondingly less water was added when the pots were brought up to weight. This observation is substantiated by the fact that in the early part of the experiment the plants in the 70-percent series grew most rapidly, but after about a month they were overtaken by the plants at the higher evel.

TABLE 1.—*Average yield of lettuce per 4-plant pot at different approximate soil moisture levels*

Soil	Yield at indicated percent of the moisture-holding capacity				
	35	45	55	70	85
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
Salinas [1]	9.31	25.18	55.71	107.18	140.74
Imperial Valley [2]	2.60	25.24	68.74	79.04	85.85

[1] Minimum significant difference between moisture levels at 5-percent point=16.60; at 1-percent=22.27.
[2] Minimum significant difference between moisture levels at 5-percent point=13.45; at 1-percent=18.04.

Table 2 shows the average weight of diseased and healthy plants grown at different moisture levels. These data are from one experiment with each of the two soils used and are more or less typical of the others. The difference between the weights of the two classes of plants was in general not significant in most experiments, but the trend was the same. It will be noticed that diseased plants grown in Salinas soil tended to be larger than the healthy ones, while the opposite was true in the Imperial Valley soil. As in the discussion on symptoms, this discrepancy can be explained on a time basis.

At the end of two of the trials with Salinas soil the plant weights were tabulated in the order in which big vein developed on the plants in a given pot. Since the data from both trials were similar, the results from only one trial are shown in table 3. These figures are based on plants in the series at 70 and 85 percent of the moisture-holding capacity, where four diseased plants per pot was not an uncommon occurrence.

TABLE 2.—*Average weight of individual diseased and healthy plants at different soil moisture levels*

Soil	Plant condition	Weight [1] at indicated percent of the moisture-holding capacity				
		35	45	55	70	85
		Grams	*Grams*	*Grams*	*Grams*	*Grams*
Salinas	Healthy	2.12**	4.82*	12.22*	17.60*	26.26
	Diseased	3.80	8.51	16.24	28.75	36.46
Imperial Valley	Healthy	.66	6.69	20.71**	26.25**	29.45**
	Diseased	.61	5.93	16.30	18.14	17.14

[1] *=difference between healthy and diseased plants significant at the 5-percent level; **=significant at the 1-percent level.

TABLE 3.—*Length of disease period in relation to weight of plants grown on Salinas soil*

Number of diseased plants	Average disease period	Average weight
	Days	Grams
17	23.2	16.0
16	17.4	20.2
12	13.6	24.8
5	11.0	14.6

Except for the last plant diseased, it is evident that the longer a plant had big vein the less its weight at harvest. In all the experiments the fourth plant to become diseased in a pot was smaller than those previously affected. There appear to be two explanations for this: (1) The rosette plants mentioned previously were much stunted, and when they became affected with big vein, it was always near the end of an experiment; (2) throughout the experiments the more vigorously growing plants developed big vein most quickly. As a result, the smallest plants were the last to become diseased, which also tended to bring the average weight of the last group down. An observation which lends some support to this explanation is the fact that when three plants in a pot had big vein, the remaining healthy plant was generally smaller than at least two of the diseased plants.

DISCUSSION

The method of adding water to the soil in pots undoubtedly would cause moisture to be present in larger amounts near the central slotted pipes, particularly in the lower moisture series. As a result, root growth at the lower levels of moisture would be more or less restricted to the center of the pot. It is possible that the small root area resulting from a limited water supply was a more important factor in reducing the amount of big vein than the direct effect of decreased moisture itself. This would be particularly true if the disease were not distributed uniformly through the soil. However, since the nature of the disease-causing agent has not been demonstrated, it is not certain whether the results obtained were due to the effect of moisture upon the host, upon the causal agent, or upon the disease complex.

The results of the present investigations show that in trials of lettuce strains for resistance to big vein it is important to maintain soil moisture at a high level not only because big vein develops in a larger number of plants when abundant water is supplied but also because the plants become affected more quickly, thus enabling the investigator to eliminate susceptible individuals early in the trials.

Observations also indicate that if soil moisture is maintained at the best level for the growth of lettuce the disease also develops well. Therefore, control of moisture in the field offers little hope of reducing big vein under commercial lettuce-growing conditions.

SUMMARY

An investigation of soil moisture in relation to the big vein disease of lettuce was made on two different soils where big vein occurred, one from near Salinas, Calif., and the other from the Imperial Valley, Calif.

The number of plants that developed big vein on these soils increased with increasing moisture. A considerable number of diseased plants were present even at moisture levels below the optimum for growth of lettuce, and a few plants had big vein when only enough water was supplied to keep them alive. The highest incidence of disease occurred in the treatments that produced the largest plants.

Plant weight increased with each increment of moisture used. The more vigorously growing individuals in each series seemed to be most readily affected with big vein, but the earlier a plant showed the disease the smaller was its final weight. With the Salinas soil, diseased plants seemed to be larger than healthy plants in the same pot, while the reverse was true with the Imperial Valley soil. The difference was attributed to a longer growing period for plants in the Imperial Valley soil.

From the results obtained it is recommended that tests of lettuce for resistance to big vein be carried out on well-watered soil. Little hope is held for reducing the number of big vein plants in commercial fields through the control of soil moisture.

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APPARENT PHOTOSYNTHESIS AND TRANSPIRATION OF PECAN LEAVES TREATED WITH BORDEAUX MIXTURE AND LEAD ARSENATE [1]

By A. J. LOUSTALOT

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INTRODUCTION

Two of the most widely used spray materials in the control of insects and diseases of the pecan are lead arsenate and bordeaux mixture. Since it has been clearly demonstrated (*6, 8, 12*) [2] that certain sprays may have a deleterious effect on the foliage of trees, it seemed advisable to determine to what extent bordeaux mixture and lead arsenate influence the photosynthesis and the transpiration of pecan leaves. Most of the literature relating to the effects of bordeaux mixture on these physiological processes is contradictory and was obtained with species other than the pecan. Lutz and Hardy (*10*) reported that pecan leaves sprayed with low-lime bordeaux mixture were more efficient in carrying on photosynthesis than unsprayed leaves. Clore (*2*), Hoffman (*8*), and others found no significant difference between the carbon dioxide intakes of bordeaux-sprayed and untreated apple leaves, whereas Southwick and Childers (*12*) found that bordeaux mixture sprayed on apple leaves caused a reduction in apparent photosynthesis. Some workers (*3, 13*) reported an increase in the rate of transpiration due to the application of bordeaux mixture, while others (*1, 4, 11*) failed to find very marked effects of this spray material on transpiration rates. Few or no data are available relating to the effects of lead arsenate on carbon dioxide assimilation or transpiration.

In this paper are presented data which show the effects of bordeaux mixture and lead arsenate applications on the rates of apparent photosynthesis and transpiration of mature leaves of the pecan, *Carya illinoensis* K. Koch (syn. *C. pecan*), at Brownwood, Tex.

MATERIALS AND METHODS

The apparatus and procedure for measuring the apparent photosynthesis were similar to those used by Heinicke and Hoffman (*7*). The apparatus consisted of six carbon dioxide absorption towers, of which two were attached to the check leaves, two were attached to the test leaves, and two served as air controls. The assimilation chambers were similar to those described by Heinicke (*5*) and were adapted for use with pecan leaves by Loustalot and Hamilton (*9*). A continuous stream of air at the rate of 2 to 2.5 liters per square centimeter of leaf area per hour (*7*) was drawn through the chambers

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[1] Received for publication September 14, 1942.
[2] Italic numbers in parentheses refer to Literature Cited, p. 19.

by mercury pumps. The water transpired by the leaves was determined by weighing, after it was absorbed by a dehydrating agent (pumice stone impregnated with sulfuric acid) through which the air was passed before it reached the carbon dioxide absorption towers. The volume of air passing through each absorption tower was accurately measured by a wet-test flowmeter.

All the experiments were conducted during the summer of 1941 with the mature leaves of a 10-year-old pecan tree of the Western variety growing near the laboratory of the United States Department of Agriculture Pecan Field Station, Brownwood, Tex. The tree was in good vigor and was carrying a full crop of nuts. The foliage, which was abundant and dark green, had been sprayed in late spring with zinc sulfate (3–100) to control pecan rosette, but at the time the experiments were started there was no visible residue.

Two pairs of mature, healthy, dark-green leaflets, comparable in position and exposure to sunlight, were selected for each experiment. One pair was designated as check and the other as test leaves before any determinations of transpiration or photosynthesis were begun. Two determinations were made each day in all the experiments—one between 9:00 a. m. and 1:15 p. m. and the other between 1:30 and 4:30 p. m. Weather observations, including temperature (°F.) and relative humidity (percent), were made at the beginning, the middle, and the end of each determination. Owing to lack of facilities for recording solar radiation, the general sky condition prevailing during each determination was estimated.

Apparent photosynthesis was calculated as the average number of milligrams of carbon dioxide assimilated per hour by 100 cm.^{2} of leaf surface. Transpiration was calculated as the average number of grams of water lost per hour by 100 cm.^{2} of leaf area. The relation in the apparent photosynthesis and transpiration was first established between the check and the test leaflets for a period of 4 to 5 days, after which the spray material was applied to the test leaflets.

Three experiments with bordeaux mixture were performed between June 27 and July 28, and two experiments with lead arsenate were conducted between September 2 and October 10. In all instances the spray material was applied to both surfaces of the test leaflets by dipping them twice into a thoroughly agitated, freshly prepared mixture and allowing the material to dry on the leaflets between immersions. The spray material was applied in the morning and had thoroughly dried on the leaves before the determinations were made.

It will be noted that in each of the five experiments the test leaves had lower rates of transpiration and apparent photosynthesis than the check leaves. This was purely accidental and has not particular significance. In setting up each experiment the two pairs of leaflets were selected, numbered, and designated test and check before any determinations were made.

EXPERIMENTS WITH BORDEAUX MIXTURE

The data for the three experiments with bordeaux mixture are presented graphically in figures 1 to 3. Since the relation between the two sets of leaflets before the spray application was fairly constant,

as shown in the figures, it was possible to obtain a good estimate of the efficiency of each set of leaves; any appreciable deviation from this established relation after the spray material was applied could be attributed to the treatment.

FIGURE 1.—The effects of a single application of bordeaux mixture on the apparent photosynthesis and transpiration of mature pecan leaves exposed to full sunlight: *A–C*, Morning determinations of carbon dioxide assimilated (*A*), of water transpired (*B*), and of the accompanying weather conditions (*C*); *D–F*, afternoon determinations of carbon dioxide assimilated (*D*), of water transpired (*E*), and of the accompanying weather conditions (*F*).

In experiment 1 (fig.1) both sets of leaflets were exposed to full sunlight during both the morning and the afternoon period except on cloudy days, when the light varied with the extent of cloudiness. Bordeaux mixture (6–2–100) was applied to the test leaflets at 8 a. m. on July 2. In the succeeding 5 days, inclusive of the day of spraying, the relation in the transpiration and the apparent photosynthetic activity between the two sets of leaflets differed very little from that established during the standardization period from June 27 to July 1. A maximum average temperature of 94° F. occurred on the second afternoon following the spray application but caused no noticeable effect on the transpiration and photosynthetic relations between the check and test leaflets. The variations in the apparent photosynthetic relation between the two pairs of leaflets on June 27 and 28 may have been due to some unbalanced internal conditions in the leaflets or to errors in experimental manipulation or determinations.

In experiment 2 (fig. 2) the procedure was similar to that in experiment 1, except that both sets of leaflets were partially shaded by adjacent foliage during both the morning and the afternoon period. Bordeaux mixture (6–2–100) was applied to the test leaflets on the morning of July 12. During the next 4 days, including the day the spray was applied, there was relatively little change in the relation in the two processes between the two sets of leaflets as compared with

FIGURE 2.—The effects of a single application of bordeaux mixture on the apparent photosynthesis and transpiration of mature pecan leaves exposed to partial sunlight: *A–C*, Morning determinations of carbon dioxide assimilated (*A*), of water transpired (*B*), and of the accompanying weather conditions (*C*); *D–F*, afternoon determinations of carbon dioxide assimilated (*D*), of water transpired (*E*), and of the accompanying weather conditions (*F*).

that during the standardization period. The temperature during this period was unusually low for the time of year.

In experiment 3 (fig. 3) the exposure of both sets of leaflets during the morning and the afternoon period was similar to that described for the leaflets used in experiment 1. This experiment differed from experiments 1 and 2 in that the test leaflets received three applications of bordeaux mixture, each on a different day, and the third application was of an 8–8–100 mixture instead of the recommended 6–2–100. The first application was made on July 20, the second on July 23, and the third on July 25. The test leaflets were so well covered with spray residue at the end of the third application that the green color of the leaflets was hardly visible. The results were more or less similar to those obtained in experiments 1 and 2—that is, the relation in the two processes between sprayed and check leaflets remained fairly constant both before and after the application of bordeaux mixture to test leaflets. The fact that the apparent photosynthesis was not affected in the leaflets covered by three spray applications indicates that the absorption of light energy was not sufficiently reduced by the spray residue to influence the rate of carbon dioxide assimilation.
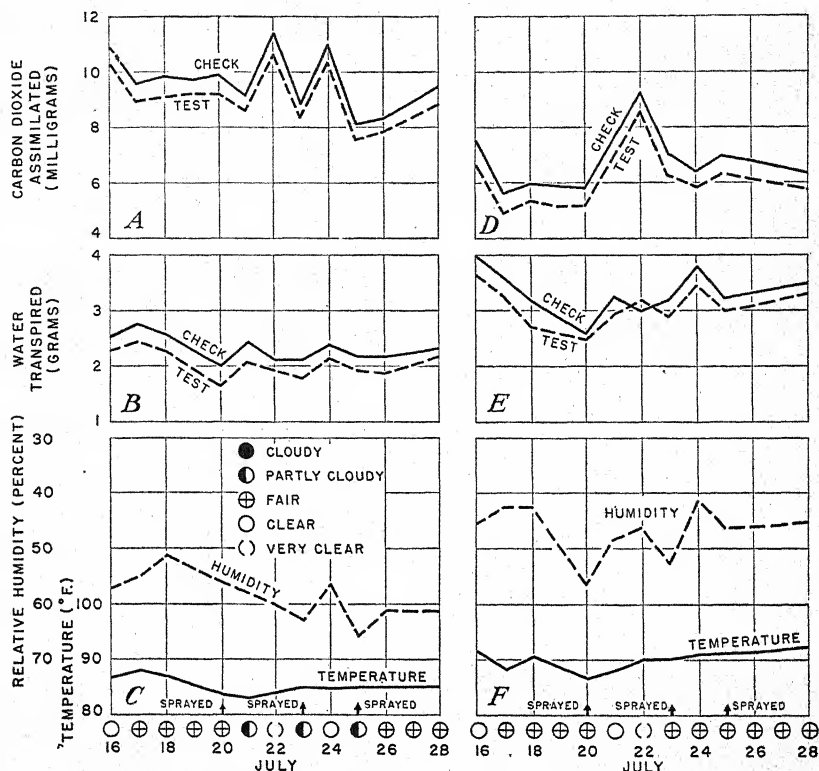
FIGURE 3.—The effects of three successive applications of bordeaux mixture on the apparent photosynthesis and transpiration of mature pecan leaves exposed to full sunlight: *A–C*, Morning determinations of carbon dioxide assimilated (*A*), of water transpired (*B*), and of the accompanying weather conditions (*C*); *D–F*, afternoon determinations of carbon dioxide assimilated (*D*), of water transpired (*E*), and of the accompanying weather conditions (*F*).

The data show that under the conditions of these experiments bordeaux mixture had little or no effect on the apparent photosynthesis and transpiration of mature pecan leaves. It will be noted from the photosynthesis and transpiration curves that small but inconsistent deviations occurred in the relation between the check and test leaflets, but these deviations fall within the limits of experimental error since they are no greater than differences between duplicate determinations from a single pair of leaflets. The deviations in duplicate determinations were usually less than 5 percent, but in a few instances they were higher, the maximum being about 14 percent. The variations in the ratios between the test and check leaflets, before and after the spray application, were usually less than 5 percent, the maximum being approximately 7 percent.

Lutz and Hardy (*10*) reported a higher photosynthetic rate for pecan leaves sprayed with low-lime bordeaux than for unsprayed leaves. However, they pointed out that the unsprayed leaves were lighter in color than those sprayed and in addition contained diseased areas. In the same paper these authors presented data indicating

that dark-green pecan leaves are more efficient in carbon dioxide assimilation than those of a lighter green color, a fact that Heinicke (6) demonstrated with apple leaves. It has also been shown (9) that even a mild infection by the fungus causing the disease known as downy spot [*Mycosphaerella caryigena* (Demaree and Cole)] markedly reduces the ability of pecan leaves to assimilate carbon dioxide. In view of these facts, it seems reasonable to assume that the beneficial effects of bordeaux mixture on the efficiency of pecan leaves observed by Lutz and Hardy may be attributed to the indirect effect on disease and the intensification of green color rather than to the direct effect of the spray material itself. It should be emphasized in this connection that the leaves used in the experiments reported herein were dark green and free from any visible disease injury, and that, therefore, any direct influence of bordeaux mixture on the two physiological processes could be determined.

EXPERIMENTS WITH LEAD ARSENATE

In the experiments with lead arsenate the same general procedure was followed as in the bordeaux mixture experiments. The first experiment was begun on September 2 and terminated on September 13, and the exposure of the two pairs of leaflets for morning and afternoon periods was similar to that described for the leaflets used in experiment 1 with bordeaux mixture. Lead arsenate (6–100) was applied to the test leaflets on three different dates, September 8, 10, and 12.

In the second experiment, started on September 29 and terminated on October 10, one spray application was made on the morning of October 6. In this case the two pairs of leaflets were located on the east side of the tree and exposed in such a way as to receive full benefit from sunlight, except when cloudy weather obscured the sun, but they were fully shaded by the adjacent foliage during the afternoon periods. No determinations were made on the afternoons of October 4 and 5.

The experimental data for the lead arsenate experiments are presented in figures 4 and 5. These data show no appreciable effect of lead arsenate applications on the rates of transpiration and carbon dioxide assimilation of pecan leaves. There was a wide variation in the average temperature during the experimental periods from September 8 to 13 (fig. 4), the maximum of 93° F. occurring in the afternoon period of September 8 and the minimum of 67° occurring during the morning period of September 9. The average relative humidity varied from a low of 34 percent during the afternoon of September 8 to a high of 96 percent during the morning of September 9. With decreases in temperature and increases in relative humidity the rate of transpiration decreased, but in neither experiment (figs. 4 and 5) was there any appreciable difference in the established ratio between the check and test leaflets. Apparent photosynthesis varied considerably with fluctuations in light intensity and other meteorological factors, but in no instance was there any marked change in the established relation in this process between sprayed and unsprayed leaflets. The minor deviations that occurred in the ratio of both transpiration and apparent photosynthesis rates in test and check leaflets are within the limits of experimental error. Therefore, appli-
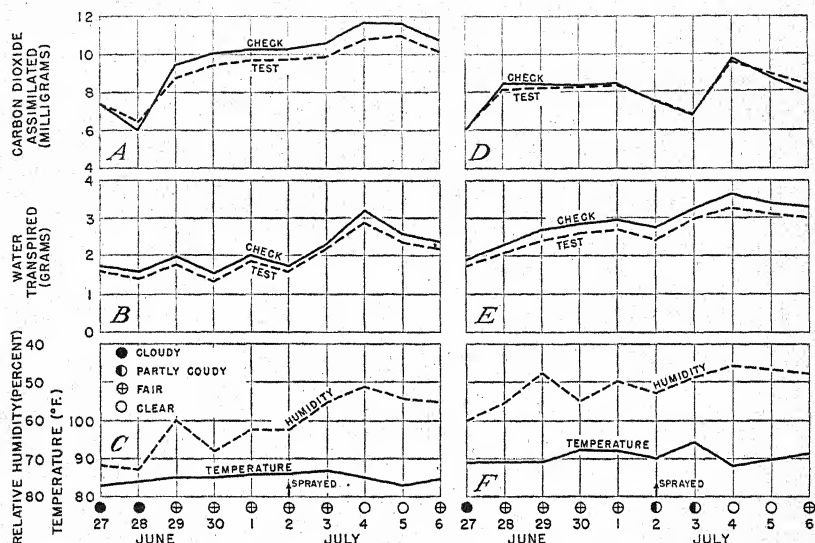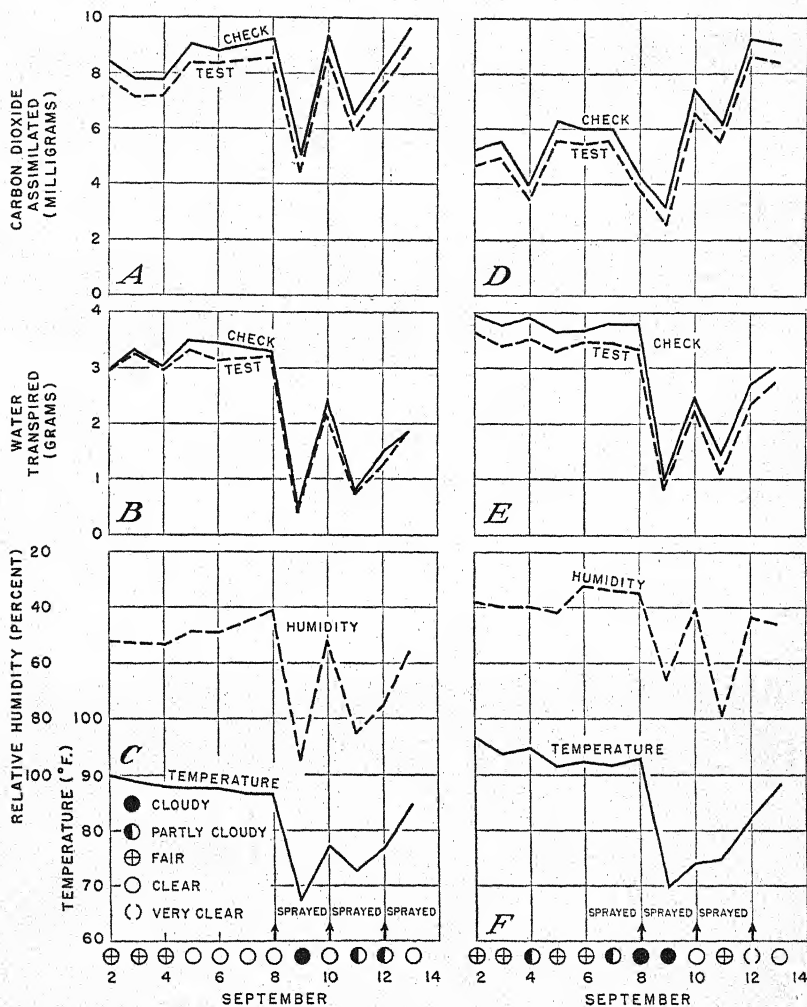
Figure 4.—The effects of three successive applications of lead arsenate on the apparent photosynthesis and transpiration of mature pecan leaves exposed to full sunlight: *A–C,* Morning determinations of carbon dioxide assimilated (*A*), of water transpired (*B*), and of the accompanying weather conditions (*C*); *D–F,* afternoon determinations of carbon dioxide assimilated (*D*), of water transpired (*E*), and of the accompanying weather conditions (*F*).

cations of lead arsenate to mature pecan leaves had no significant effect on carbon dioxide assimilation or transpiration.

The fluctuations in the apparent photosynthesis from day to day of both test and check leaflets apparently were due to changes in environmental conditions, especially light, while fluctuations in transpiration seemed to follow changes in temperature and relative humidity.
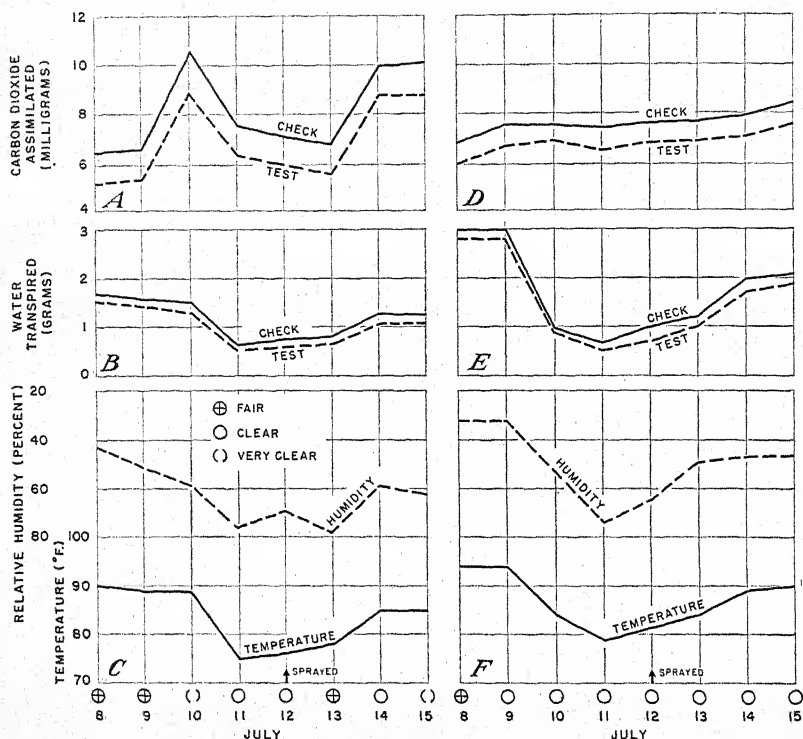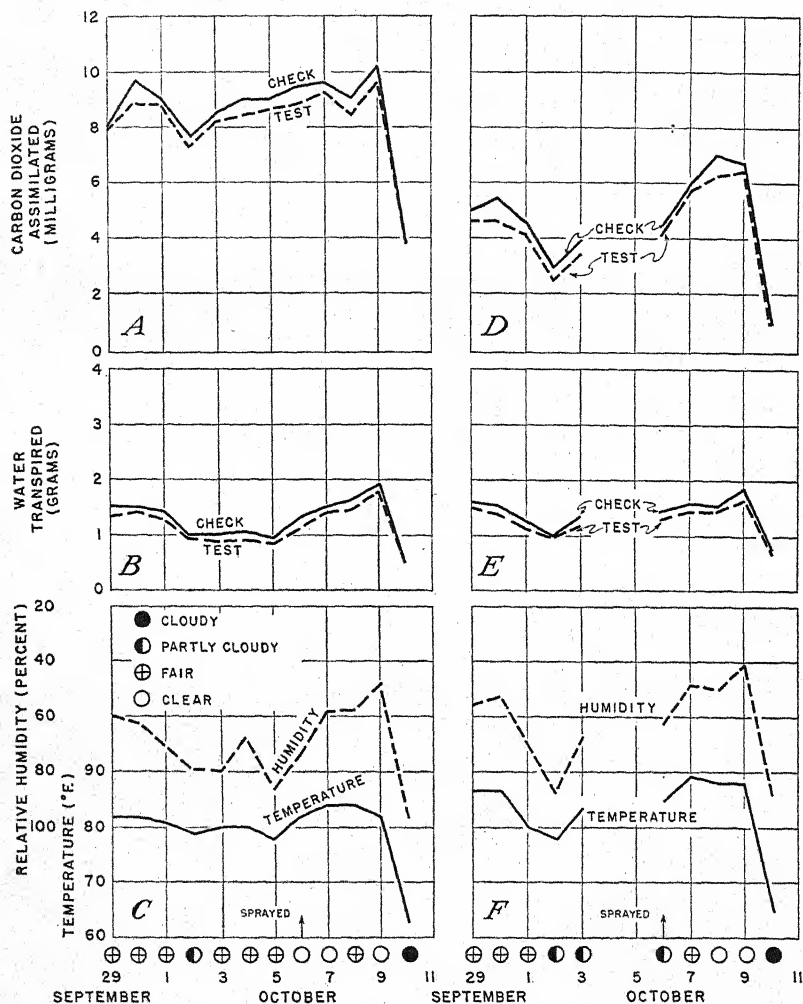
FIGURE 5.—The effects of a single application of lead arsenate on the apparent photosynthesis and transpiration of mature pecan leaves exposed to full sunlight in the morning and full shade in the afternoon: A–C, Morning determinations of carbon dioxide assimilated (A), of water transpired (B), and of the accompanying weather conditions (C); D–F, afternoon determinations of carbon dioxide assimilated (D), of water transpired (E), and of the accompanying weather conditions (F).

SUMMARY

Studies were made to determine the effects of bordeaux mixture and lead arsenate applications on the rates of the apparent photosynthesis and transpiration of mature pecan leaves on a 10-year-old pecan tree during the 1941 season. Three experiments were conducted with bordeaux mixture during the period from June 27 to July 28, and two similar experiments with lead arsenate were carried on between September 2 and October 10.

No appreciable effects on the apparent photosynthesis or transpiration were obtained by applications of either bordeaux mixture or lead arsenate to mature pecan leaves. The same results were obtained from one or three applications of the spray materials even though the leaves receiving three applications were so well covered that the green color was scarcely visible.

Wide fluctuations occurred in rates of apparent photosynthesis and transpiration from day to day and during the morning and afternoon periods of the same day, but apparently these were due largely to changes in meteorological conditions. The rates of apparent photosynthesis were affected primarily by fluctuations in light intensity, while transpiration rates seemed to follow changes in temperature and relative humidity.

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CYTOLOGICAL AND GENETIC ANALYSIS OF CHROMOSO-MAL ASSOCIATION AND BEHAVIOR DURING MEIOSIS IN HEXAPLOID TIMOTHY (PHLEUM PRATENSE)[1]

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INTRODUCTION

A knowledge of the type of chromosomal association occurring during meiosis is important not only for an understanding of the origin of the species but also for the intelligent planning of a breeding program. In American timothy (*Phleum pratense* L.), a hexaploid with $2n=42$, evidence regarding the kind of chromosome pairing is contradictory. The experiments reported in this paper were designed to furnish further information on this subject.

REVIEW OF LITERATURE

Gregor (*5*)[2] and Gregor and Sansome (*6*) reported synthesis of a hexaploid type by spontaneous doubling in the hybrid *Phleum pratense* $(2n=14) \times P.\ alpinum\ (2n=28)$, which was cross-fertile with ordinary hexaploid *P. pratense*. On the basis of these results and the behavior in a natural hybrid which he supposed to be *P. pratense* $(2n=14) \times P.\ pratense\ (2n=42)$, Müntzing (*8*) concluded that American timothy was an allohexaploid. Later, however, Nordenskiöld (*16*) reported 14 bivalents in F_1 *P. pratense* $(2n=14) \times P.\ pratense\ (2n=42)$, Müntzing and Prakken (*10*) found 28 bivalents in 63-chromosome timothy plants obtained from twin seedlings, and Myers (*12*) observed a maximum of 12 bivalents in F_1 plants of *P. pratense* $(2n=42) \times P.\ subulatum\ (2n=14)$. In polyhaploid $(2n=21)$ plants obtained from twin seedlings of hexaploid timothy, Nordenskiöld (*17*) found 7 bivalents plus 7 univalents. These results were in agreement with the hypothesis that at least 2 of the 3 genomes of the reduced complement in timothy were homologous. On the other hand, Müntzing (*8*) and Nordenskiöld (*16*) stated that multivalents were not found in meiosis in the hexaploid, while Müntzing and Prakken (*10*) reported that multivalents were very rare, if they occurred at all. Myers (*12*) found a low frequency of quadrivalents in the 1 plant studied. The apparently contradictory evidence of homology between genomes and absence of multivalents was discussed in detail by Müntzing and Prakken (*10*), and they raised the question whether each of the bivalents was formed always from the same 2 chromosomes or whether 2 or 3 bivalents were formed by random pairing of 4 or 6 homologues.

There is a paucity of critical genetic evidence from hexaploid timothy. Barker and Hayes (*1*), Proytchoff, according to Horsfall (*7*), and Myers and Chilton [3] obtained typical monohybrid ratios for resistance to rust. Clarke (*3*) explained the occurrence of chlorophyll-deficient seedlings among inbred progenies by the assumption of triplicate factors. Using similar material, Wexelsen (*20*) obtained results that he interpreted on the basis of single, duplicate, and triplicate factors, assuming that the species was an allohexaploid. In two families, however, he obtained results that suggested the possibility of tetrasomic inheritance.

MATERIAL AND METHODS

For the cytological studies, plants were selected from different sources as follows: (1) Three plants from three seed collections in New Jersey, (2) one plant from Cornell strain No. 1777, (3) three F_1 hybrids involving parents from seed collected in New Hampshire and Vermont, and (4) three plants from open-pollinated seed produced on a plant selection from S48, pasture-hay strain from the Welsh Plant Breeding Station, Aberystwyth, Wales.

The plants were grown in the greenhouse during the winter of 1940–41, and microsporocyte material was killed and fixed in acetic alcohol (1 part glacial acetic acid to 3 parts absolute alcohol). All data were collected and the photomicrographs made from fresh acetocarmine smear slides.

For the genetic studies, five unrelated plants were selected that were known from previous tests to be heterozygous for a factor or factors conditioning albino seedlings. Selfed seed from these plants and from their first inbred-generation (I^1) progenies were germinated in Petri dishes, and the seedlings were classified for green versus albino.

EXPERIMENTAL RESULTS

CYTOLOGICAL INVESTIGATIONS

DIAKINESIS

The type of chromosomal association was determined at diakinesis for 7 of the plants (table 1). Of the 102 sporocytes examined, only 2 had 21 bivalents; all the others had quadrivalents, sexivalents, or both, in addition to bivalents. The frequency of quadrivalents in the sprocytes ranged from 1 to 7, the average being 2.9 to 4.9 quadrivalents per sporocyte among the 7 plants. It is worthy of note that the range in quadrivalent frequency is similar' to that found among plants of *Dactylis glomerata* L. (*14*, *15*) and of tetraploid *Lolium perenne* L. in which there are 4 genomes of 7 chromosomes each.[4] Sexivalents were found in 33 of the 102 sporocytes; of these, 26 had 1 sexivalent (fig. 1, *A* and *B*), 6 had 2, and 1 had 3. In 1 of the sporocytes with a sexivalent the nucleolar chromosomes apparently were involved (fig. 1, *B*), whereas in the sporocyte with 3 sexivalents, none consisted of nucleolar chromosomes. The results indicate that at least 4 of the 7 chromosomes are capable of pairing with their homologous or homeologous chromosomes from the other 5 genomes. Sexivalents were found in 1 or more

[3] MYERS, W. M., and CHILTON, S. J. P. [Unpublished data obtained at the U. S. Regional Pasture Research Laboratory.]
[4] MYERS, W. M. [Unpublished data obtained at the U. S. Regional Pasture Research Laboratory.]
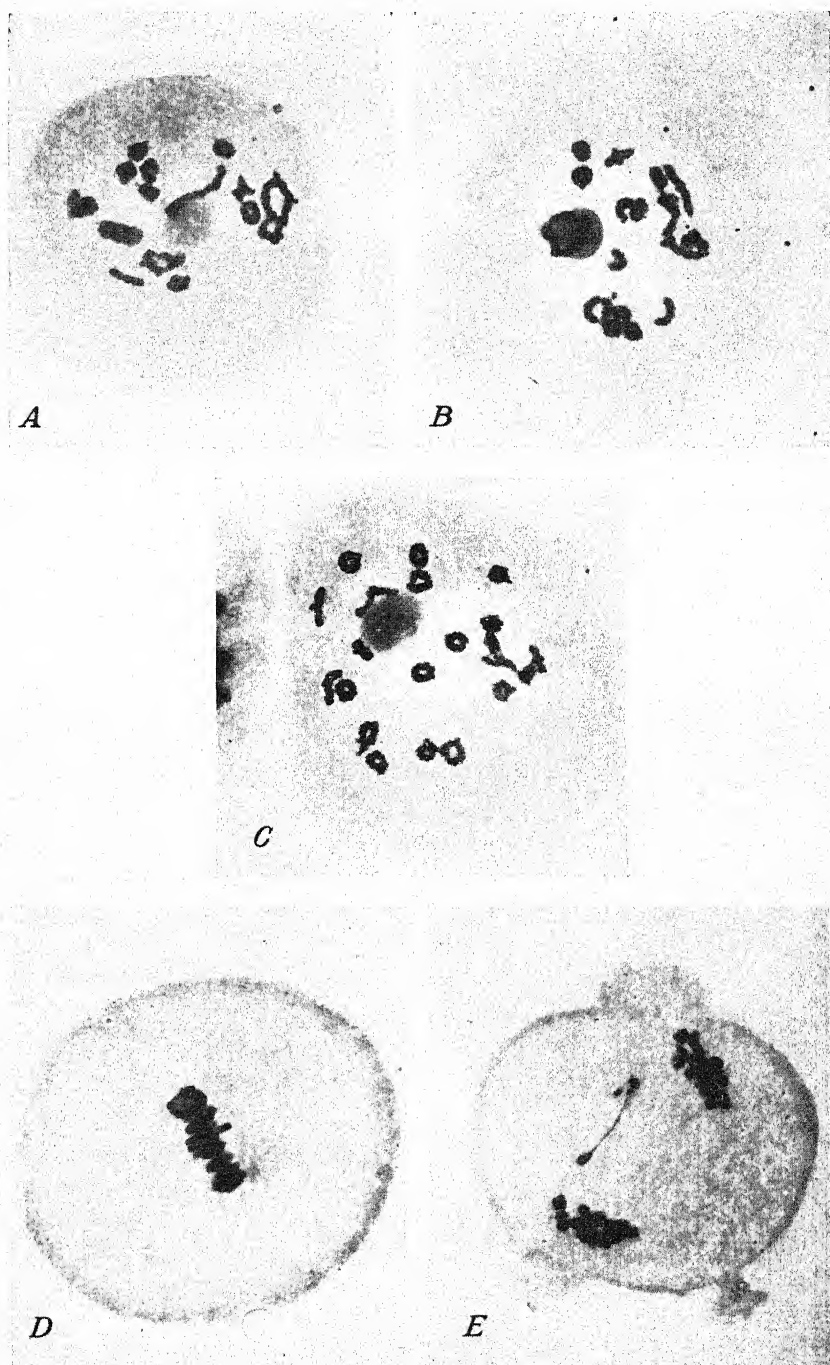
FIGURE 1.—*A*, Diakinesis in *Phleum pratense* with one sexivalent plus quadrivalents and bivalents; *B*, diakinesis with a sexivalent lying on the nucleolus; *C*, diakinesis showing secondary association of the bivalents; *D*, metaphase I with ring quadrivalent; *E*, late anaphase I with dicentric chromatid bridge and acentric fragment.

sporocytes in 6 plants, while in the other plant only 9 sporocytes were examined.

TABLE 1.—*Frequency of bivalents, quadrivalents, sexivalents, and half-chiasmata per chromosome at diakinesis in 7 plants of Phleum pratense*

Chromosome groups per cell or half-chiasmata per chromosome (number)	Cells, chromosomes, or half-chiasmata in plant No.—							Total cells
	Ti 49 (4)	Pot 1441	Pot 1443	Ti 11 (8)	Ti 14 (5)	Ti 16 (5)	Ti 46 (9)	
	Number	Number	Number	Number	Number	Number	Number	Number
Chromosomes per cell:								
2_{II}	1	0	0	0	1	0	0	2
$19_{II}+1_{IV}$	3	1	1	0	0	0	0	5
$17_{II}+2_{IV}$	5	1	1	0	3	0	0	10
$15_{II}+3_{IV}$	8	4	1	2	2	1	0	18
$13_{II}+4_{IV}$	5	3	1	2	3	1	0	15
$11_{II}+5_{IV}$	2	0	1	0	1	4	0	8
$9_{II}+6_{IV}$	1	0	2	0	2	1	4	10
$7_{II}+7_{IV}$	0	0	0	0	0	1	0	1
$16_{II}+1_{IV}+1_{VI}$	1	0	1	0	0	0	0	2
$14_{II}+2_{IV}+1_{VI}$	3	0	0	1	1	0	1	6
$12_{II}+3_{IV}+1_{VI}$	4	0	1	0	0	0	1	6
$10_{II}+4_{IV}+1_{VI}$	1	0	2	0	0	0	1	4
$8_{II}+5_{IV}+1_{VI}$	2	0	0	0	0	2	1	5
$6_{II}+6_{IV}+1_{VI}$	0	0	0	0	0	0	3	3
$15_{II}+0_{IV}+2_{VI}$	0	0	1	0	0	0	0	1
$11_{II}+2_{IV}+2_{VI}$	0	0	1	0	0	0	0	1
$9_{II}+3_{IV}+2_{VI}$	0	0	0	0	0	1	1	2
$7_{II}+4_{IV}+2_{VI}$	0	0	0	0	0	0	1	1
$5_{II}+5_{IV}+2_{VI}$	0	0	0	0	0	0	1	1
$8_{II}+2_{IV}+3_{VI}$	0	0	1	0	0	0	0	1
Total cells	36	9	14	5	13	11	14	102
Average bivalents per cell	14.2	15.0	12.5	14.0	14.2	10.3	8.5	
Average quadrivalents per cell	2.9	3.0	3.1	3.2	3.3	4.8	4.9	
Average sexivalents per cell	.3	0	.8	.2	.1	.4	.9	
Half-chiasmata per chromosome in—								
Bivalents	1.64	1.88	1.75	1.77	1.90	1.56	1.70	
Quadrivalents	1.70	1.94	1.85	1.91	1.86	1.82	1.79	
Sexivalents	1.88		1.94	1.67	2.00	1.83	1.87	
Average half-chiasmata per chromosome	1.67	1.90	1.80	1.81	1.89	1.69	1.76	

In most sporocytes there was a tendency for the bivalents to lie together in pairs (fig. 1, *C*). In many cases it was impossible to determine whether a configuration consisted of four chromosomes associated as a quadrivalent or of two bivalents lying so close together that they touched, and such doubtful cases were recorded as two bivalents. Müntzing (*8*) observed cases of secondary association at metaphase I in *Phleum pratense*. It is the opinion of the author that the association of bivalents in pairs at diakinesis in this material was a relic of multivalent pairing at prophase rather than a secondary attraction of bivalents during diakinesis.

Among the seven plants, the average frequency of half-chiasmata per chromosome ranged from 1.67 to 1.90 (table 1). It is apparent from an examination of table 1 that chiasma frequency was not closely related to bivalent frequency among the different plants. If chiasma frequency were the principal factor conditioning variations among plants in the formation of multivalents, a negative correlation of chiasma with bivalent frequency would have been expected. Instead, a correlation coefficient of +0.437 was obtained. With

five degrees of freedom, this value is not significant statistically (*4, table VA*); nevertheless, the results suggest that factors other than chiasma frequency have played a major role in conditioning the differences among plants in the formation of multivalents.

Although other factors appear to have been more important than chiasma frequency in determining differences among plants, there is evidence, nevertheless, that chiasma frequency was a limiting factor in multivalent formation.   Within plants, the number of half-chiasmata per chromosome was higher for the chromosomes associated as quadrivalents than for those associated as bivalents in all but one plant. Likewise, the chiasma frequency per chromosome in the sexivalents was higher than that in the quadrivalents in all but one plant.   Upcott (*19*) has pointed out that two opposing forces are involved in the relationship of chiasma frequencies in bivalents and multivalents.   In those cases where chiasma frequency is not a limiting factor in multivalent formation, the number of chiasmata per chromosome should be lower in multivalents than in bivalents because of the less complete pairing resulting from exchanges of partners in prophase.   On the other hand, where chiasma frequency is limiting, the chromosomes associated as multivalents represent a selected group with a higher frequency of chiasmata per chromosome.   The plants used in this study apparently fall in the latter category.

METAPHASE I

With the technique used, metaphase I was unsuited for determination of bivalent and multivalent frequency.   The chromosomes were contracted and clumped together on the equatorial plate.   Nevertheless, quadrivalents could be distinguished with certainty in numerous sporocytes (fig. 1, *D*).   Also, it was possible at this stage to determine the frequency of sporocytes with unpaired chromosomes.   The average percentage of sporocytes with univalents ranged from 1.1 to 14.3 percent for the 10 plants (table 2).

TABLE 2.—*Percentages of metaphase I sporocytes with univalents, of anaphase I sporocytes with lagging and dividing univalents and with dicentric chromatid bridges and acentric fragments, and of quartets with micronuclei*

Plant No.	Metaphase I		Anaphase I			Quartets	
				Sporocytes with—			
	Cells	Sporocytes with univalents	Cells	Lagging univalents	Bridge+fragment	Cells	With micronuclei
	Number	*Percent*	*Number*	*Percent*	*Percent*	*Number*	*Percent*
Ti 46 (9)	89	1.1	21	0	4.8	114	3.5
Ti 14 (5)	194	4.1	35	0	0	110	0
Pot 1443	124	4.8	23	4.4	4.4	51	9.8
Ti 11 (8)	124	4.8	56	1.8	0	101	0
Ti 7 (9)	109	6.4	29	0	0	81	3.7
Ti 49 (4)	93	6.5	29	3.4	0	99	8.1
Ti 160 (1)	239	9.2	54	3.7	7.4	87	1.2
Pot 1439	52	13.5	75	6.7	5.0	70	8.6
Pot 1441	50	14.0	26	23.1	3.8	66	15.1
Ti 16 (5)	126	14.3	28	17.8	0	75	6.7

ANAPHASE I

In 3 plants, no lagging univalents were observed in the 21, 29, and 35 sporocytes examined. In the other 7 plants, 1 or more sporocytes had lagging univalents that were undergoing equational division, the frequency of such sporocytes ranging from 1.8 to 23.1 percent for the different plants (table 2).

In five of the plants, 3.8 to 7.4 percent of the anaphase I sporocytes had a dicentric chromatid bridge accompanied by an acentric fragment (fig. 1, *E*), indicating that these plants were heterozygous for a chromosomal aberration, probably an inversion. In the plants in which bridges and fragments were not observed, the numbers of sporocytes examined were not sufficient to preclude the possibility of their occurrence.

QUARTETS

The frequency of quartets with micronuclei ranged from 0 to 15.1 percent for the 10 plants and appeared to be correlated with the frequency of laggards at anaphase I (table 2).

GENETIC INVESTIGATIONS

CALCULATED RATIOS

In a hexaploid species, such as *Phleum pratense*, 3 different types of chromosomal behavior may be obtained: (1) The species may be an allohexaploid, in which case 21 bivalents will be formed regularly; (2) the species may be an autohexaploid in which the homologous chromosomes of the 6 genomes pair at random to form bivalents, quadrivalents, and sexivalents; or (3) there may be 4 genomes of one kind and 2 of another, so that the chromosomes of the former will pair at random, forming bivalents and quadrivalents, while the chromosomes of the latter genomes regularly form bivalents. In addition, there may be any intermediate condition between these extremes, depending upon the degree of differentiation of the chromosomes of the different genomes.

On the first assumption, duplicate and triplicate factors segregating in monohybrid, dihybrid, and trihybrid ratios would be expected. If the second condition obtains, hexasomic ratios would result (table 3). In the third type, various combinations of tetrasomic and disomic (monohybrid) ratios would be obtained. The expected ratios on this hypothesis for all genotypes from which segregation would occur in I_1 or I_2 are given in table 4. In this case the gene *Aa* is assumed to be located in chromosomes of the four homologous genomes, while the gene *Bb*, a duplicate of *Aa*, is located in chromosomes of the remaining two genomes. There is also the possibility that no duplicate of *Aa* occurred in the remaining two genomes, in which case only tetrasomic ratios would be expected. In plants in which multivalent pairing occurs, the genetic ratios are conditioned by position of the gene with reference to the centromere as well as by the relative number of dominant and recessive alleles that are present. The effects of the position of the gene have been illustrated in detail in an earlier paper *(13)*, and these effects have been taken into account in the calculation of the ratios presented in tables 3 and 4.

TABLE 3.—*Calculated ratios in I_1 and I_2 from parents of different genotypes on the assumption of random pairing of the homologous chromosomes from 6 genomes*

ON THE ASSUMPTION OF CHROMOSOME ASSORTMENT

Genotype of parent	Ratio in I_1	Number of dominant I_1 phenotypes showing indicated segregation in I_2			
		∞ :0	399:1	24:1	3:1
A_6	Not segregating	All	0	0	0
A_5a_1	do	All	0	0	0
A_4a_2	do	18.0	6.0	1.0	0
A_3a_3	399:1	6.6	9.1	5.6	1.0
A_2a_4	24:1	1.0	6.0	11.0	6.0
A_1a_5	3:1	0	0	1.0	2.0

ON THE ASSUMPTION OF CHROMATID ASSORTMENT

Genotype of parent	Ratio in I_1	Number of dominant I_1 phenotypes showing indicated segregation in I_2				
		∞ :0	3,024:1	120:1	14. 4:1	2.4:1
A_6	Not segregating	All	0	0	0	0
A_5a_1	do	360.0	105.0	18.0	1.0	0
A_4a_2	3,024:1	40.8	46.7	29.2	8.3	1.0
A_3a_3	120:1	1.1	3.2	4.7	3.2	1.0
A_2a_4	14.4:1	1.0	8.0	28.0	44.8	31.4
A_1a_5	2.4:1	0	1.0	18.0	105.0	216.0

TABLE 4.—*Calculated ratios in I_1 and I_2 from parents of different genotypes on the assumption of random association of homologous chromosomes of 4 genomes and pairing of homologous chromosomes of remaining 2 genomes*

ON THE ASSUMPTION OF CHROMOSOME ASSORTMENT

Genotype of parent	Ratio in I_1	Number of dominant I_1 phenotypes showing indicated segregation in I_2				
		∞ :0	143:1	35:1	15:1	3:1
A_3a_1 Bb	Not segregating	13.0	2.0	1.0	0	0
A_3a_1 bb	do	3.0	0	1.0	0	0
A_2a_2 Bb	143:1	6.3	3.6	1.8	1.6	1.0
A_2a_2 bb	35:1	1.0	0	2.2	0	1.0
A_1a_3 Bb	15:1	4.0	2.0	1.0	4.0	4.0
A_1a_3 bb	3:1	0	0	1.0	0	2.0
a_4 Bb	3:1	1.0	0	0	0	2.0

ON THE ASSUMPTION OF CHROMATID ASSORTMENT

Genotype of parent	Ratio in I_1	Number of dominant I_1 phenotypes showing indicated segregation in I_2						
		∞ : 0	3,135:1	783:1	86.1:1	20.8:1	12.9:1	3 :1	2.5:1
A_3a_1 Bb	3, 135:1	729.5	360.0	180.0	174.0	87.0	24.0	1	12.0
A_3a_1 bb	783:1	9.4	0	15.0	0	7.2	0	0	1.0
A_2a_2 Bb	86. 1:1	12.4	5.3	2.7	9.1	4.6	5.3	1	2.7
A_2a_2 bb	20.8:1	1.0	0	5.3	0	9.1	0	0	5.3
A_1a_3 Bb	12.9:1	32.8	2.0	1.0	14.5	7.2	30.0	18.8	15.0
A_1a_3 bb	2.5:1	1.0	0	24.0	0	174.0	0	0	360.0
a_4 Bb	3:1	1.0	0	0	0	0	0	2	0

Observed Ratios

In the I_1 generation of two of the plants, namely, Ti 2 (11) and Ti 6 (15), the ratios of green to albino seedlings were 546:19 and 626:24, respectively (table 5). As revealed by the χ^2 test, the deviations of these ratios from either 15:1 or 63:1 were too great to be attributed to the errors of random sampling. On the other hand, the fit to the calculated ratios 35:1, 20.8:1, and 24:1 was satisfactory. Therefore, the results in I_1 were in agreement with either of the following hypotheses: (1) That the plants were duplex for the gene located in the four homologous genomes, that the duplicate gene in the remaining two genomes was homozygous recessive or absent, and that either chromosome (35:1 ratio) or chromatid (20.8:1 ratio) assortment obtained (table 4), or (2) that the plants were duplex with six homologues pairing and disjoining at random (table 3).

In the I_2 generation of Ti 2 (11), 23 lines averaging 99.3 seedlings per line were classified (table 6). The character used in these investigations was lethal, and consequently all I_2 lines reported here and later in the paper were progenies of normal I_1 plants. Homozygous recessive I_2 lines were neither expected nor obtained. The fit of the I_2 lines to the calculated dihybrid ratio was on the border line of statistical significance (P lies between 0.02 and 0.05). When χ^2 was determined for each segregating family and the values summed, total χ^2 gave P of less than 0.01. The goodness of fit of observed to calculated trihybrid ratios (not shown in table 6) was less satisfactory than the fit to the dihybrid ratios. Therefore, the results in I_2 support the conclusion drawn from I_1 that dihybrid and trihybrid ratios were not obtained in the progeny of Ti 2 (11).

TABLE 5.—*Obtained ratios and goodness of fit to various calculated ratios in the first inbred generation of 5 plants of Phleum pratense*

Plant No.	Obtained ratio		Value of P of χ^2 for fit to indicated ratio							
	Green	Albino	143 : 1	120 : 1	86.1 : 1	63 : 1	35 : 1	24 : 1	20.8 : 1	15 : 1
Ti 2 (11)	546	19	<0.01	<0.01	<0.01	<0.01	0.30–0.50	0.30–0.50	0.10–0.20	<0.01
Ti 6 (15)	626	24	<.01	<.01	<.01	<.01	.10– .20	.70– .80	.20– .30	<.01
Ti 16 (5)	575	4	>.99	.70–0.80	.30–0.50	.05–0.10	<.01	<.01	<.01	<.01
Ti 15 (5)	632	0	.02–0.05	.02	<.01	<.01	<.01	<.01	<.01	<.01
Ti 11 (8)	557	0	.02– .05	.02– .05	.01– .02	<.01	<.01	<.01	<.01	<.01

On the basis of the hypothesis of duplex constitution of four homologous genomes with the duplicate gene in the remaining genomes homozygous recessive (A_2a_2bb), the fit of I_2 lines to calculated was good when either chromosomal or chromatid assortment was assumed. Furthermore, total χ^2 gave P of 0.10 to 0.20 on the hypothesis of chromosomal assortment, but P was less than 0.01 for fit of observed to the calculated for random chromatid segregation. The fit also was good of the observed ratio of I_2 lines to the expected ratio on the hypothesis that the parent plant was duplex with hexasomic chromosome pairing (A_2a_4). Total χ^2 gave P between 0.02 and 0.05 in this case.

TABLE 6.—*Obtained and calculated ratios and goodness of fit to various hypotheses in the second inbred generation of 4 plants of Phleum pratense*

Parent No.	Hypothetical genotype	Number of I_2 lines—			Value of P for—	
		Not segregating	Segregating		I_2 lines	Total χ^2
			35:1, 24:1, 20.8:1, or 15:1	3:1 or 2.5:1		
		[1] 7.00	[1] 12.00	[1] 4.00		
	$A_2a_2\,bb$ [2]	5.88	11.77	5.35	0.70–0.80	0.10–0.20
Ti 2 (11)	$A_2a_2\,bb$ [3]	7.00	10.11	5.89	.50– .70	<.01
	A_2a_4 [2]	6.72	10.56	5.76	.50– .70	.02– .05
	$Aa\ Bb$	10.71	6.12	6.12	.02– .05	<.01
		[1] 3.00	[1] 11.00	[1] 8.00		
	$A_2a_2\,bb$ [2]	5.63	11.26	5.12	.20– .30	<.01
Ti 6 (15)	$A_2a_2\,bb$ [3]	6.68	9.64	5.62	.10– .20	.30– .50
	A_2a_4 [2]	6.44	10.12	5.52	.20– .30	.10– .20
	$Aa\ Bb$	10.29	5.88	5.88	<.01	.02– .05
		[1] 16.00	[1] 9.00			
	$A_3a_2\,bb$ [2]	18.75	6.25		.20– .30	.50– .70
	$A_3a_1\,bb$ [3]	19.01	5.18	.72	.10– .20	.05– .10
Ti 15 (5)	$A_2a_2\,Bb$ [2]	17.30	5.94	1.75	.10– .20	[4]
	$A_2a_2\,Bb$ [3]	11.83	11.02	2.15	.10– .20	[5]
	A_3a_3 [2]	17.58	6.27	1.12	.20– .30	.10– .20
		[1] 14.00	[1] 9.00			
	$A_3a_1\,bb$ [2]	17.25	5.75		.20– .30	.50– .70
	$A_3a_1\,bb$ [3]	17.20	5.08	.70	.10– .20	.02– .05
Ti 11 (8)	$A_2a_2\,Bb$ [2]	16.34	4.95	1.65	.05– .10	[4]
	$A_2a_2\,Bb$ [3]	10.81	10.07	1.96	.20– .30	[5]
	A_3a_3 [2]	16.17	5.77	1.03	.10– .20	.10– .20

[1] Obtained ratio.
[2] Calculated on the basis of chromosome assortment.
[3] Calculated on the basis of random chromatid assortment.
[4] Total χ^2 cannot be calculated since both 35 : 1 and 15 : 1 ratios were expected and these cannot always be distinguished with the numbers of plants used.
[5] Total χ^2 cannot be calculated since 86.1 : 1, 20.8 : 1, and 12.9 : 1 ratios were expected and these cannot always be distinguished with the numbers of plants used.

The results in I_2 of Ti 6 (15) were similar to those for Ti 2 (11) (table 6). In this case, 22 I_2 lines averaging 125.6 seedlings per line were classified. The observed ratios did not fit satisfactorily either the hypothesis of dihybrid or trihybrid inheritance. In contrast to Ti 2 (11), the fit was satisfactory to the ratios expected from the genotype A_2a_2bb with chromatid assortment, but total χ^2 gave P of less than 0.01 on the assumption of chromosomal assortment. The fit was equally good for the hypothesis of duplex factors with hexasomic segregation (A_2a_4).

In the I_1 generation of Ti 15 (5) and Ti 11 (8), 632 and 557 seedlings, respectively, were classified and all were normal. Failure to obtain recessive seedlings in such large populations would occur very rarely if duplicate or triplicate factors with disomic inheritance were involved unless one or more of the genes were homozygous. Segregation in some of the I_2 lines in each family furnished evidence of heterozygosity for factors conditioning albino seedlings. Among the progeny of Ti 15 (5), 25 I_2 lines with an average of 79.4 seedlings per line were studied (table 6). The results substantiated the conclusion drawn from I_1 that neither dihybrid nor trihybrid ratios were involved. On the basis of the χ^2 test, it may be concluded that the fit was satisfactory of observed ratios to those expected from the genotype A_3a_1bb with chromosomal or chromatid segregation, the genotype A_2a_2Bb with chromosomal or chromatid segregation, or the genotype A_3a_3 with chromosomal assortment. Chromatid assortment from

the genotype A_2a_2Bb seems to be an unlikely explanation since in I_1 the deviation from expected on that basis gave P of less than 0.01.

The results obtained in I_2 of Ti 11 (8), in which 23 lines with 88.8 seedlings per line were studied, were similar to the results with Ti 15 (5). In one of the 9 segregating lines in this family, the observed ratio (127:14) deviated more than was expected by chance ($P<0.01$) from a 35:1, 20.8:1, or 24:1 ratio. The fit of this observed ratio to 15:1 was satisfactory ($P=0.05$ to 0.10). No ratio of this kind is expected in I_2 from the genotype A_3a_3 with chromosome assortment but ratios of 15:1 are expected in I_2 from the genotype A_2a_2Bb with chromosomal assortment, and ratios of 12.9:1 are expected in I_2 from A_2a_2Bb with chromatid segregation. Occasional I_2 lines segregating 15:1 also might be expected from a parent of the genotype A_2a_2bb since the occurrence of sexivalents indicated that all six homologous or homeologous chrosomes may pair. In such cases, a dominant allele could be transferred by crossing-over to one of the chromosomes normally carrying bb.

In the I_1 of Ti 16 (5) a ratio of 575 green : 4 albino was obtained. The deviations of this ratio from 143:1, 120:1, 86.1:1, or 63:1 were not statistically significant (table 5). In I_2, there were 5 lines not segregating to 18 segregating. None of the segregating lines showed a ratio approximating 3:1. The deviation from the expected on the basis of trihybrid inheritance was too great to be attributed to chance. Also, P was less than 0.01 for fit of the observed ratio to that calculated for the genotype A_2a_2Bb with chromatid segregation. Chi square for fit of observed to calculated for the genotype A_3a_3 with chromatid segregation gave P of 0.10 to 0.20.

DISCUSSION

The cytological results obtained in these investigations differ from those reported previously for hexaploid *Phleum pratense* in the high quadrivalent frequency and in the occurrence of sexivalents. In one plant, Myers (12) reported a low frequency of quadrivalents. However, the chiasma frequency of the plant was low, probably accounting for the low quadrivalent frequency. The disagreement with the results by Nordenskiöld (16) and Müntzing and Prakken (10) may have resulted from the different conditions under which the plants were grown or from differences in material. The plants studied by these authors probably were of European origin, whereas seven of the plants used in this investigation were from local collections in the northeastern part of the United States.

The plants used in these investigations had varying proportions of metaphase I sporocytes with univalents, anaphase I with laggards, and quartets with micronuclei. Such meiotic irregularities usually are expected in species with multivalent pairing. The differences among plants suggest the possibility of reducing the amount of irregularity in the species by selection. Similar differences among plants of *Dactylis glomerata* have been reported by Myers and Hill (14, 15).

The incidence of dicentric bridges and acentric fragments at anaphase I is in agreement with the reports of Müntzing (9), Myers and Hill (14, 15), Myers (11, 12), and Östergren (18), of the frequent

occurrence of chromosomal aberrations among plants of naturally cross-pollinated species.

Genetic results reported for *Phleum pratense* have been limited primarily to the first segregating generation. Geneticists have recognized generally that tests in the second segregating generation (I_2 or F_3) are much more sensitive than I_1 or F_2 tests   Myers (*12*, *13*) has emphasized particularly the inadequacy of the I_1 test for distinguishing between disomic and polysomic inheritance. The types of chromosomal association that might be expected in a hexaploid have been outlined previously in this report. The high quadrivalent frequency observed in these plants provides cytological evidence against the hypothesis of allohexaploidy, and the genetic results were completely in agreement with the cytological in that regard so far as the genes studied in these experiments are concerned. The possibility cannot be ignored, of course, that the chromosomes may be differentiated to varying degrees so that one set will usually pair as three bivalents while the others associate at random in fours or sixes. Critical evidence on this point can be obtained only when marker genes are available for most or all of the chromosomes.

The quadrivalent frequency in the plants investigated was similar to that obtained in *Dactylis glomerata* (*14*, *15*), autotetraploid *Lolium perenne*,[5] unpublished, and several other autotetraploid grasses (*14*). This result together with the low incidence of sexivalents might be interpreted to indicate that there were four genomes of one kind and two of another instead of six homologous genomes. On this hypothesis, the occasional sexivalents could be explained on the basis of incomplete differentiation of chromosomes of the two genomes from those of the four. However, such cytological evidence is not critical. The two alternative hypotheses can be evaluated adequately only by genetic data. In the present investigations, the numbers of I_2 lines and the numbers of plants within lines were too small to afford a sensitive genetic test of the hypotheses. In addition to the use of larger populations, I_3 tests of selected I_2 lines would be valuable for such a test.

The results obtained in these investigations are of considerable importance to the plant breeder. In an allohexaploid, only monohybrid dihybrid, and trihybrid ratios are expected for simply inherited characters. Homozygosity of the dominant allele in one of the three genomes is effective in preventing further phenotypic segregation, while lines segregating in a monohybrid ratio will contain only plants that are homozygous and plants that produce progenies again segregating in a 3 : 1 ratio. In polyploids with six homologous genomes or with four genomes of one kind and two of another, the genetic results are much more complex. This fact is evident from the calculated ratios presented in tables 3 and 4. In a polyploid of either of these types, two, or in some cases three, generations of progeny tests would be required to prove that a plant was homozygous for any particular factor.

In naturally cross-pollinated species, such as *Phleum pratense*, inbreeding is the most effective method of obtaining uniformity for important characters. According to the calculations of Bartlett and Haldane (*2*), the rate of approach to homozygosity is expected to be

[5] See footnote 4.

very slow in autopolyploids. Therefore, more generations of inbreeding will be required in *P. pratense* than in an allohexaploid to attain the same degree of homozygosity. On the other hand, this characteristic of autopolyploids would be an advantage in the use of hybrids involving inbred lines for commercial production, since the reduction in vigor in advanced generations of the hybrids should be less rapid than with diploids or allopolyploids.

SUMMARY

In the plants of hexaploid *Phleum pratense L.* that were studied, the chromosomes at diakinesis were associated as bivalents, quadrivalents, and sexivalents. Among seven plants, the average number of quadrivalents per sporocyte ranged from 2.9 to 4.9 and the average number of sexivalents from 0 to 0.9. In any sporocyte the maximum number of quadrivalents observed was 7 and of sexivalents 3. Chiasma frequency was not correlated with multivalent frequency in these plants.

The plants had varying percentages of univalents at metaphase I, lagging and dividing chromosomes, dicentric bridges, and acentric fragments at anaphase I, and micronuclei in the quartets.

Genetic data from the I_1 and I_2 generations of five plants were in agreement with the cytological results in that dihybrid and trihybrid ratios were not obtained, indicating that the chromosomes did not pair regularly as particular bivalents. The genetic data were insufficient to afford critical evidence as to whether there are six homologous genomes or four genomes of one kind and two of another in *Phleum pratense*.

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EFFECT OF HEAT TREATMENT AND OIL EXTRACTION ON THE UTILIZATION AND DIGESTIBILITY OF SOYBEAN PROTEIN BY LAMBS [1]

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INTRODUCTION

Soybeans and soybean oil meal have become increasingly important as livestock feeds in this country. With the recent great increase in the acreage of soybeans because of the demand for soybean oil, the availability of soybean oil meal for livestock feeding will be even greater. The utilization of this soybean protein to the best advantage is of considerable importance to the livestock industry.

Various experiments with nonruminants, such as pigs, chickens, and rats, have shown that both raw soybeans and soybean oil meals that have not been well cooked furnished protein of poor quality or low value when fed as the principal source of protein (*2, 5, 9, 4, 11, 12, 13, 15, 16, 20, 21*.)[3] On the other hand, these experiments have shown that well-cooked soybean oil meals furnish good-quality protein, and that heat treatment of raw soybeans, or further heat treatment of insufficiently cooked soybean oil meals, results in marked improvement in the efficiency of the protein.

The improvement of the soybean protein by heat treatment was shown by some increase in digestibility and considerable increase in efficiency. In general, for rats the heated soybean protein had a digestibility from 3 to 7 percent greater than that of raw soybean protein (*2, 4, 16, 20*). The biological values for rats reported by Hayward et al. (*2*) averaged 41 for raw soybean protein and 51 for heated soybean protein when each was fed at an 18-percent level of protein. Mitchell and Beadles (*4*) give biological values of 49 for raw soybean protein and 67 for heated soybean protein when fed to rats at a 10-percent level of protein, as reported by the Illinois station (*4, pp. 93–95*).

Certain investigators have found that cystine supplements raw soybean protein, thereby greatly improving its value in rations for rats (*9, 4, 16*). Mitchell and Beadles (*4*) found that cystine also supplements cooked soybean protein. However, when the cystine deficiencies of both the raw and heated soybean proteins were corrected, heated soybeans were still superior for rats. These investigators concluded that heating makes not only cystine but also probably certain other amino acids more available to rats.

Few nitrogen-balance experiments or digestion trials to measure the effect of heat treatment on the nutritive value and digestibility of soybean protein have been conducted with ruminants. Scharrer

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and Nebelsiek (*14*) found that for sheep the coefficients of digestibility of the various nutrients of steamed soybeans were higher than those for raw soybeans. Hamilton and Kammlade (*3, pp. 73–76*), at the Illinois station, found no difference in the digestibility of the protein of soybeans and soybean oil meal when fed to lambs.

As summarized by Morrison (*10*), feeding experiments have shown that raw soybeans and well-cooked soybean oil meal are of about equal value as protein supplements to rations for dairy cattle and sheep. For all beef cattle, except fattening calves, soybeans have been found equal in feeding value to soybean oil meal. There has been little or no difference for ruminants in the value of the protein furnished by soybean oil meals prepared by different methods. In Indiana experiments King [4] found no advantage in cooking soybeans for fattening cattle.

OBJECTS OF EXPERIMENTS

The objects of the experiments reported in this paper were (1) to determine the effect of heat treatment on the digestibility and nutritive value of soybean protein for lambs, and (2) to determine whether or not the addition of soybean oil to a soybean oil meal ration, in an amount equal to that contained in a soybean ration, would affect the ability of lambs to digest or utilize protein.

EXPERIMENTAL PROCEDURE

Nitrogen-balance experiments were conducted on wether lambs of good mutton type. The urine and feces were collected daily by means of metabolism cages during 10-day collection periods. All preliminary or intervening periods were at least 10 days in length. The general procedure and methods of analysis were practically the same as those followed in the earlier work reported by this station (*18, 19*).

The data on digestibility are expressed as apparent digestibility and as estimated true digestibility. The efficiency of nitrogen utilization is expressed as percentage storage of total nitrogen, percentage storage of digested nitrogen, and biological value. No direct determinations of endogenous nitrogen losses in the urine and metabolic nitrogen losses in the feces were made on these lambs. Experimental work conducted at this station (*8*) has indicated that low-nitrogen periods of feeding, necessary for making these determinations, cause unduly high nitrogen utilization by lambs when subsequently fed the test rations. Therefore, as in earlier work (*6, 7*), average values for metabolic nitrogen losses and endogenous nitrogen losses, as determined at this station on comparable lambs, were used. The average values were 0.037 gm. of endogenous nitrogen daily per kilogram of body weight and 0.55 gm. of metabolic nitrogen per 100 gm. of dry-matter intake. The soundness of these average values has been strengthened by a recent paper of Harris and Mitchell (*1*). Using a similar low-nitrogen basal ration, they report average values for lambs of 0.033 gm. of endogenous nitrogen daily per kilogram of body weight and 0.555 gm. of metabolic nitrogen per 100 gm. of dry-matter intake.

[4] KING, F. G. Ind. Agr. Expt. Sta. Mimeo. Rpts. (Unpublished data.)

EXPERIMENT 1

The four soybean feeds used in experiment 1 were raw soybeans, soybean flakes, solvent-process soybean oil meal, and heat-treated solvent-process soybean oil meal. These feeds were furnished by a Corn Belt soybean oil mill, and were from the same batch of soybeans.

The raw soybeans were of good quality, and presumably were typical of soybeans marketed in the Corn Belt. By analysis, they contained 35.1 percent of crude protein and 18.9 percent of ether extract.

The soybean flakes were a product resulting from the initial processing of soybeans in the solvent-extraction method. The beans were first dried at a maximum temperature of 180° F. for approximately 20 minutes. Then the dried beans were cracked, heated to a maximum temperature of 165° F. for 15 minutes, and flaked. Therefore, the soybean flakes as fed in this experiment were unextracted and had received only sufficient heat treatment for efficient flaking. They were light in color and had the same crude-protein and ether-extract content as the raw soybeans. This product should not be confused with extracted soybean flakes, which receive more heat treatment.

The solvent-extracted soybean oil meal was produced by the commercial process of extracting soybeans with hexane. It received only sufficient heat for drying the beans, flaking them, and removing the hexane. Frequently, such meal has been referred to as white soybean oil meal or raw soybean oil meal. In the extraction process, the soybean flakes described above were held at a temperature of approximately 125° F. for 30 minutes, and the extracted residue was then heated to a maximum of 200° for 20 minutes to drive off the solvent. The resulting meal contained 44.6 percent of crude protein and 0.9 percent of ether extract.

The heat-treated solvent-process soybean oil meal was produced by further heating or "toasting" of the extracted meal for 70 minutes at a maximum temperature of approximately 250° F. This meal was light brownish or tan in color. The crude-protein content was 42.1 percent and the ether-extract content was 0.9 percent.

The low-nitrogen basal ration used in this experiment was the low-nitrogen ration for lambs developed at this station for use in determining biological values of proteins. It was composed of purified cellulose, starch, brown sugar, corn oil, minerals, and wheat straw. The total protein content averaged about 1.0 percent. Numerous nitrogen-balance trials have shown this basal ration to be entirely inadequate to support either nitrogen equilibrium or maintenance of body weight.

The composition of each ration as fed is given in table 1.

Each of the soybean products was included in sufficient amounts to furnish 10 percent total protein in its respective ration. The lambs were fed twice daily and each ration was fed as a complete mixture. The order in which each lamb was fed each of the four rations was varied in such a manner as to make an orderly rotation.

The data of experiment 1 are presented in table 2 and the values for the digestibility and the utilization of the nitrogen are summarized in table 3.

TABLE 1.—*Composition of the rations fed in experiment 1*

	Ration containing—			
Ingredients	Raw ground soybeans	Soybean flakes	Solvent-process soybean oil meal without special heat treatment	Solvent-process soybean oil meal with special heat treatment
	Percent	*Percent*	*Percent*	*Percent*
Soybean product	28.50	28.50	22.43	23.75
Cellulose	9.00	9.00	9.00	9.00
Wheat straw	25.00	25.00	25.00	25.00
Starch	17.25	17.25	19.28	18.62
Sugar	17.25	17.25	19.29	18.63
Corn oil			2.00	2.00
Mineral [1]	3.00	3.00	3.00	3.00
Average total protein	10.41	10.48	10.96	11.09
Average fat	5.77	5.77	2.58	2.59

[1] The mineral mixture was composed of 40 percent ground limestone, 40 percent steamed bonemeal, and 20 percent salt.

TABLE 2.—*Nitrogen-balance data showing digestibility and utilization of nitrogen in experiment 1.*[1]

GROUND SOYBEAN RATION

Number of lamb	Weight of lamb			Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Total nitrogen stored	Apparent digestibility	True digestibility (estimated)	Digested nitrogen stored	Biological value
	Initial	Final	Average										
	Kg.	*Kg.*	*Kg.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	
1	27.6	27.3	27.4	8,000	7,368.8	134.19	56.55	57.06	15.3	57.5	87.8	26.7	61
2	30.9	31.1	31.0	8,000	7,356.4	133.96	58.61	52.10	17.4	61.1	91.4	28.4	62
3	27.8	27.3	27.6	6,000	5,486.7	99.91	36.53	41.41	22.0	58.6	88.9	37.6	70
4	22.5	23.1	22.8	7,000	6,405.0	116.64	53.26	57.03	5.4	51.1	81.4	10.6	53
5	28.5	27.8	28.2	8,000	7,330.0	133.48	72.64	53.82	5.3	59.7	90.0	8.8	48
6	27.8	28.0	27.9	8,000	7,293.6	132.82	70.85	38.26	17.8	71.2	100.0	25.1	54
7	32.3	32.3	32.3	8,000	7,293.6	132.82	62.50	48.91	16.1	63.2	93.5	25.5	59
8	31.9	32.6	32.2	8,000	7,318.8	133.28	65.62	40.14	20.6	69.9	100.0	29.5	60

SOYBEAN FLAKE RATION

Number of lamb	Initial	Final	Average	Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Total nitrogen stored	Apparent digestibility	True digestibility	Digested nitrogen stored	Biological value
1	29.2	29.9	29.6	8,000	7,274.0	132.97	62.09	46.89	18.0	64.7	94.9	27.9	59
2	34.9	35.0	35.0	8,000	7,300.8	133.46	61.95	38.46	24.8	71.2	100.0	34.8	63
3	27.8	28.4	28.1	8,000	7,377.2	134.86	55.66	56.22	17.0	58.3	88.5	29.2	62
4	25.3	25.8	25.6	8,000	7,327.2	133.94	54.21	56.32	17.5	58.0	88.1	30.2	62
5	24.1	24.4	24.2	6,000	5,501.1	100.56	58.44	34.52	7.6	65.7	95.9	11.5	49
6	24.1	25.3	24.7	7,000	6,427.4	117.49	61.38	43.53	10.7	63.0	93.2	17.0	52
7	30.4	31.0	30.7	8,000	7,319.2	133.80	60.20	47.81	19.3	64.3	94.5	30.0	61
8	30.7	30.7	30.7	8,000	7,274.0	132.97	60.93	51.47	15.5	61.3	91.5	25.2	59

SOYBEAN OIL MEAL RATION

Number of lamb	Initial	Final	Average	Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Total nitrogen stored	Apparent digestibility	True digestibility	Digested nitrogen stored	Biological value
1	28.1	29.0	28.6	8,000	7,340.4	140.35	70.13	38.36	22.7	72.7	100.0	31.2	58
2	32.4	33.2	32.8	8,000	7,393.2	139.45	71.01	50.22	13.1	64.0	92.9	20.4	55
3	31.8	32.3	32.0	8,000	7,293.2	139.45	63.61	39.37	26.2	71.8	100.0	36.4	63
4	28.5	29.9	29.2	8,000	7,376.0	141.03	53.91	41.72	32.2	70.4	99.3	45.7	69
5	26.2	26.1	26.2	8,000	7,337.2	140.29	60.05	49.37	22.0	64.8	93.7	34.0	62
6	26.2	27.4	26.8	8,000	7,306.4	139.70	69.83	49.96	14.2	64.2	93.1	22.2	54
7	24.6	25.2	24.9	6,000	5,520.9	105.56	53.33	35.94	15.4	66.0	94.8	23.4	56
8 [2]	27.3	25.0	26.2	5,103	4,699.6	89.86	48.80	40.26	.9	55.6	84.1	1.2	48

HEAT-TREATED SOYBEAN OIL MEAL RATION

Number of lamb	Initial	Final	Average	Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Total nitrogen stored	Apparent digestibility	True digestibility	Digested nitrogen stored	Biological value
1	25.5	25.8	25.6	6,000	5,524.8	106.90	58.10	35.61	12.3	66.7	95.2	18.5	52
2	28.4	28.6	28.5	7,000	6,455.0	124.90	64.06	35.85	20.0	71.3	99.8	28.1	57
3	30.4	30.9	30.6	8,000	7,278.4	140.84	73.93	44.84	14.9	68.2	96.7	23.0	54
4	26.4	27.5	27.0	8,000	7,248.8	140.26	59.44	45.54	25.2	67.5	96.1	37.2	63
5	28.5	29.7	29.1	8,000	7,248.8	140.26	53.22	44.13	32.7	70.7	99.2	46.3	69
6	29.1	30.0	29.6	8,000	7,388.4	142.97	58.30	47.63	25.9	66.7	95.2	38.9	65
7	27.2	28.4	27.8	8,000	7,367.2	142.56	64.43	43.97	24.0	69.2	97.7	34.6	61
8	28.7	29.2	29.0	8,000	7,440.0	143.96	54.48	50.73	26.9	64.8	93.3	41.6	67

[1] Totals represent 10-day experimental periods.
[2] Data not included in summary of results because of low feed intake and loss of weight.

TABLE 3.—*Summary of digestibility and utilization of nitrogen in experiment 1*

Item	Data for lamb No. —								Average [1]
	1	2	3	4	5	6	7	8	
Ground soybean ration:									
Apparent digestibility_____percent__	57.5	61.1	58.6	51.1	59.7	71.2	63.2	69.9	61.5±1.57
True digestibility_____do____	87.8	91.4	88.9	81.4	90.0	100.0	93.5	100.0	91.6±1.49
Total nitrogen stored_____do____	15.3	17.4	22.0	5.4	5.3	17.8	16.1	20.6	15.0±1.51
Digestible nitrogen stored_____do____	26.7	28.4	37.6	10.6	8.8	25.1	25.5	29.5	24.0±2.31
Biological value_____	61	62	70	53	48	54	59	60	58 ±1.60
Soybean flake ration:									
Apparent digestibility_____percent__	64.7	71.2	58.3	58.0	65.7	63.0	64.3	61.3	63.3±1.03
True digestibility_____do____	94.9	100.0	88.5	88.1	95.9	93.2	94.5	91.5	93.3± .94
Total nitrogen stored_____do____	18.0	24.8	17.0	17.5	7.6	10.7	19.3	15.5	16.3±1.25
Digestible nitrogen stored_____do____	27.9	34.8	29.2	30.2	11.5	17.0	30.0	25.2	25.7±1.84
Biological value_____	59	63	62	62	49	52	61	59	58 ±1.23
Soybean oil meal ration:									
Apparent digestibility_____percent__	72.7	64.0	71.8	70.4	64.8	64.2	66.0	------	67.7± .96
True digestibility_____do____	100.0	92.9	100.0	99.3	93.7	93.1	94.8	------	96.3± .86
Total nitrogen stored_____do____	22.7	23.1	26.2	32.2	22.0	14.2	15.4	------	20.8±1.79
Digestible nitrogen stored_____do____	31.2	20.4	36.4	45.7	34.0	22.2	23.4	------	30.5±2.33
Biological value_____	58	55	63	69	62	54	56	------	60 ±1.38
Heat-treated soybean oil meal ration:									
Apparent digestibility_____percent__	66.7	71.3	68.2	67.5	70.7	66.7	69.2	64.8	68.1± .52
True digestibility_____do____	95.2	99.8	96.7	96.1	99.2	95.2	97.7	93.3	96.6± .52
Total nitrogen stored_____do____	12.3	20.0	14.9	25.2	32.7	25.9	24.0	26.9	22.7±1.59
Digestible nitrogen stored_____do____	18.5	28.1	23.0	37.2	46.3	38.9	34.6	41.6	33.5±2.28
Biological value_____	52	57	54	63	69	65	61	67	61 ±1.47

[1] The error calculated is the probable error of the mean.

The apparent digestibility of the nitrogen averaged 61.5 percent for the raw soybean ration, 63.3 percent for the soybean flake ration, 67.7 percent for the solvent-process soybean oil meal ration, and 68.1 percent for the toasted soybean oil meal ration. These data show an appreciably lower digestibility for the raw soybean protein and the soybean flake protein than for the protein in either of the soybean oil meal rations. The average values for the estimated true digestibility of the nitrogen were also higher for the two rations containing the meals than for the rations containing either the raw soybeans or the soybean flakes. Further heat treatment of the solvent-process soybean oil meal resulted in little or no improvement in digestibility.

The lambs stored nearly the same percentage of the total nitrogen intake when fed either the heat-treated soybean oil meal ration or the ration with the soybean oil meal without special heat treatment. The average retention was 22.7 percent and 20.8 percent, respectively. Also, the lambs stored nearly the same percentage of the total nitrogen intake when fed either the ground soybean ration or the soybean flake ration, but the storage on each was lower than when the soybean oil meals were fed.

The percentage retention of the digested nitrogen was greatest by the lambs while they were fed the heat-treated soybean oil meal ration, or 33.5 percent. The lambs stored 30.5 percent, or only slightly less, when fed the ration with soybean oil meal without special heat treatment. The retention of digested nitrogen was appreciably less by the lambs when fed the ground soybeans and the soybean flakes, being 24.0 percent and 25.7 percent, respectively.

There was little difference in the average biological values of the four rations. The values, however, were in the same order as the values obtained on the percentage retention of digested nitrogen which did not consider the endogenous and metabolic nitrogen losses.

EXPERIMENT 2

Nitrogen-balance experiments were conducted on another group of eight lambs in a further study of the effect.of heat treatment and oil extraction on the digestibility and utilization of soybean protein. Ground raw soybeans, solvent-process soybean oil meal, and solvent-process soybean oil meal with special heat treatment, similar in all respects to the products used in experiment 1, were employed. The soybean flake ration was not tested in this series, as it was believed that the first experiment had amply demonstrated that the nitrogen of soybean flakes had about the same digestibility and utilization as the nitrogen in raw soybeans. Instead, a ration was fed in which soybean oil was added with heat-treated soybean oil meal in the amount needed to bring the total fat content up to that of the ground soybean ration. Refined soybean oil was used for this purpose as lambs failed to consume well a ration containing the needed amounts of crude soybean oil. The low-nitrogen basal ration in this experiment was the same as that used in the first experiment, except that soybean oil replaced corn oil in the basal ration.

The percentage composition of the rations fed is shown in table 4.

TABLE 4.—*Composition of the rations fed in experiment 2*

Ingredients	Ration containing—			
	Raw ground soybeans	Solvent-process soybean oil meal without special heat-treatment	Solvent-process soybean oil meal with special heat-treatment	Solvent-process soybean oil meal with special heat-treatment plus soybean oil
	Percent	*Percent*	*Percent*	*Percent*
Soybeans or soybean oil meal	28.22	23.60	21.94	21.94
Cellulose	9.0	9.0	9.0	9.0
Wheat straw	25.0	25.0	25.0	25.0
Starch	17.39	19.20	19.53	17.84
Sugar	17.39	19.20	19.53	17.84
Soybean oil		2.0	2.0	5.38
Mineral [1]	3.0	3.0	3.0	3.0
Average total protein	10.50	11.44	11.25	11.31
Average fat	5.94	2.50	2.56	5.94

[1] The mineral mixture was composed of 40 percent ground limestone, 40 percent steamed bonemeal, and 20 percent salt.

The detailed results of experiment 2 are given in table 5 and the data on digestibility and utilization of the nitrogen are summarized in table 6.

The results of this experiment check closely with the results obtained in the first experiment. In general, values for both the digestibility and the utilization of the nitrogen tended to be higher than those in experiment 1.

The apparent digestibility and the estimated true digestibility of the nitrogen in the raw soybean ration averaged lower than for the other rations. The apparent digestibility of the nitrogen averaged 64.3 percent as compared with 70.4 percent for the solvent-process soybean oil meal ration and 73.1 for the treated soybean oil meal ration. There was no significant difference in the digestibility of the nitrogen between the ration containing the solvent-process meal without special heat treatment and the ration with the toasted meal.

The addition of soybean oil to the ration containing heat-treated soybean oil meal, in the amount needed to give the same fat content as in the raw soybean ration, had a slightly depressing effect on the digestibility of the nitrogen. This difference was not sufficient to be statistically significant.

TABLE 5.—*Nitrogen-balance data showing digestibility and utilization of nitrogen in experiment 2* [1]

GROUND SOYBEAN RATION

Number of lamb	Weight of lamb			Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Total nitrogen stored	Apparent digestibility	True digestibility (estimated)	Digestible nitrogen stored	Biological value
	Initial	Final	Average										
	Kg.	Kg.	Kg.	Gm.	Gm.	Gm.	Gm.	Gm.	Pct.	Pct.	Pct.	Pct.	
1	32.8	32.7	32.8	7,867	7,237.6	131.65	58.58	46.60	20.1	64.6	95.0	31.1	63
2	32.8	33.2	33.0	8,000	7,375.2	134.15	63.89	55.81	10.8	58.4	88.7	18.4	56
3	30.4	31.6	31.0	7,000	6,469.4	117.68	50.33	30.08	31.7	74.4	42.6	—	67
4	27.8	28.0	27.9	7,000	6,469.4	117.68	48.92	45.82	19.5	61.1	91.4	31.9	64
5	30.2	30.0	30.1	8,000	7,376.8	134.18	57.86	46.88	21.9	65.1	95.4	33.7	64
6	27.5	27.4	27.4	7,000	6,444.2	117.22	52.53	42.16	19.2	64.0	94.4	30.0	62
7	25.2	25.9	25.6	7,000	6,429.5	116.95	45.47	45.12	22.5	61.4	91.8	36.7	66
8	25.9	25.7	25.8	5,650	5,189.5	94.40	37.14	32.40	26.3	65.7	96.0	40.1	70

SOYBEAN OIL MEAL RATION

Number of lamb	Initial	Final	Average	Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Total nitrogen stored	Apparent digestibility	True digestibility	Digestible nitrogen stored	Biological value
1	30.6	31.2	30.9	7,095	6,523.1	130.27	63.04	34.42	25.2	73.6	100.0	34.2	60
2	31.2	32.0	31.6	8,000	7,355.2	146.88	57.74	45.14	30.0	69.3	96.9	43.2	68
3	34.4	36.0	35.2	8,000	7,382.4	147.43	64.92	34.13	32.8	76.8	100.0	42.7	65
4	30.7	30.7	30.7	8,000	7,376.0	147.30	67.80	47.08	22.0	68.0	75.7	32.4	60
5	26.5	27.7	27.1	7,000	6,435.8	128.52	47.66	47.02	26.3	63.4	91.1	41.5	68
6	23.9	24.4	24.2	5,650	5,209.9	104.04	48.45	28.09	26.4	73.0	100.0	36.2	62
7	27.1	27.2	27.2	7,000	6,454.7	128.90	58.41	40.49	23.3	68.6	96.2	33.9	61
8 [2]	26.0	25.8	25.9	5,249	4,832.2	96.50	44.22	11.10	42.7	88.5	98.4	46.6	67

HEAT TREATED SOYBEAN OIL MEAL RATION—LOW FAT

Number of lamb	Initial	Final	Average	Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Total nitrogen stored	Apparent digestibility	True digestibility	Digestible nitrogen stored	Biological value
1	27.3	27.7	27.5	7,000	6,396.6	125.76	60.23	35.20	24.1	72.0	100.0	33.5	60
2	26.7	27.7	27.2	7,000	6,442.8	126.66	64.57	32.16	23.6	74.6	100.0	31.7	57
3	32.4	33.8	33.1	7,000	7,370.4	144.90	54.68	35.17	38.0	75.7	100.0	50.2	71
4	29.6	30.5	30.0	8,000	7,268.8	142.90	53.86	38.56	35.3	73.0	100.0	48.4	70
5	29.1	29.0	29.0	7,000	6,476.4	127.33	53.20	36.85	29.3	71.3	99.5	41.2	66
6	26.1	26.8	26.4	7,000	6,476.4	127.33	48.80	36.07	33.4	71.7	99.8	46.5	69
7	31.2	31.4	31.3	7,640	6,941.7	136.47	62.43	41.56	23.8	69.5	97.6	34.2	62
8	30.3	31.1	30.7	8,000	7,337.6	144.26	65.99	32.87	31.5	77.2	100.0	40.8	62

HEAT TREATED SOYBEAN OIL MEAL RATION—HIGH FAT

Number of lamb	Initial	Final	Average	Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Total nitrogen stored	Apparent digestibility	True digestibility	Digestible nitrogen stored	Biological value
1	28.6	29.2	28.9	7,000	6,493.2	127.14	54.88	39.50	25.8	68.9	97.1	37.4	64
2	29.3	30.0	29.6	7,000	6,493.2	127.14	44.59	38.72	34.5	69.5	97.7	49.6	73
3	29.1	29.6	29.4	7,000	6,433.0	125.96	52.80	33.42	31.6	73.5	100.0	42.9	67
4	26.0	27.7	26.8	7,000	6,482.0	126.92	41.35	36.43	38.7	71.3	99.5	54.3	75
5	31.0	31.8	31.4	8,000	7,369.6	144.30	70.40	36.17	26.2	74.9	100.0	34.9	59
6	28.9	29.7	29.3	7,000	6,014.4	117.76	49.44	33.09	29.9	71.9	100.0	41.6	67
7	30.0	30.3	30.2	8,000	7,366.4	144.23	57.86	49.05	25.9	66.0	94.2	39.2	66
8	26.9	27.8	27.4	7,000	6,448.4	126.26	41.55	34.24	40.0	72.9	100.0	54.8	75

[1] Total represent 10-day experimental periods.
[2] Data not included in summary of results.

The percentage of the total nitrogen stored by the lambs also averaged lower for the soybean ration. The average for this ration was 21.5 percent as compared with 26.6 percent for the soybean oil meal ration without special heat treatment, 29.9 percent for the

toasted meal ration, and 31.6 percent for the toasted meal high-fat ration. The percentage of digested nitrogen stored, uncorrected for endogenous and metabolic nitrogen losses, averaged 33.1, 37.7, 40.8, and 44.3, respectively.

TABLE 6.—*Summary of digestibility and utilization of nitrogen in experiment 2*

Item	Data for lamb No.—								Average [1]
	1	2	3	4	5	6	7	8	
Ground soybean ration:									
Apparent digestibility_____percent__	64.6	58.4	74.4	61.1	65.1	64.0	61.4	65.7	64.3±1.13
True digestibility_____do___	95.0	88.7	100.0	91.4	95.4	94.4	91.8	96.0	94.1± .82
Total nitrogen stored_____do___	20.1	10.8	31.7	19.5	21.9	19.2	22.5	26.3	21.5±1.44
Digestible nitrogen stored_____do___	31.1	18.4	42.6	31.9	33.7	30.0	36.7	40.1	33.1±1.77
Biological value_____	63	56	67	64	64	62	66	70	64 ± .98
Soybean oil meal ration:									
Apparent digestibility_____percent__	73.6	69.3	76.8	68.0	63.4	73.0	68.6	------	70.4±1.13
True digestibility_____do___	100.0	96.9	100.0	95.7	91.1	100.0	96.2	------	97.1± .83
Total nitrogen stored_____do___	25.2	30.0	32.8	22.0	26.3	26.4	23.3	------	26.6± .96
Digestible nitrogen stored_____do___	34.2	43.2	42.7	32.4	41.5	36.2	33.9	------	37.7±1.17
Biological value_____	60	68	65	60	68	62	61	------	63 ± .91
Heat-treated soybean oil meal ration—low fat:									
Apparent digestibility_____percent__	72.0	74.6	75.7	73.0	71.1	71.7	69.5	77.2	73.1± .61
True digestibility_____do___	100.0	100.0	100.0	100.0	99.1	99.8	97.6	100.0	99.6± .20
Total nitrogen stored_____do___	24.1	23.6	38.0	35.3	29.3	33.4	23.8	31.5	29.9±1.34
Digestible nitrogen_____do___	33.5	31.7	50.2	48.4	41.2	46.5	34.2	40.8	40.8±1.71
Biological value_____	60	57	71	70	66	69	62	62	65 ±1.23
Heat-treated soybean oil meal ration—high fat:									
Apparent digestibility_____percent__	68.9	69.5	73.5	71.3	74.9	71.9	66.0	72.9	71.1± .68
True digestibility_____do___	97.1	97.7	100.0	99.5	100.0	100.0	94.2	100.0	98.6± .50
Total nitrogen stored_____do___	25.8	34.5	31.6	38.7	26.2	29.9	25.9	40.0	31.6±1.36
Digestible nitrogen stored_____do___	37.4	49.6	42.9	54.3	34.9	41.6	39.2	54.8	44.3±1.82
Biological value_____	64	73	67	75	59	67	66	75	68 ±1.36

[1] The error calculated is the probable error of the mean.

There was somewhat less difference in the biological values than in the values for the uncorrected storage of digested nitrogen. The average values were 64, 63, 65, and 68, for the ground soybeans, solvent meal, heat-treated meal, and the heat-treated meal plus soybean oil, respectively.

A further comparison of the soybean ration and the solvent-process soybean oil meal ration was made as a part of a third experiment, as yet unpublished, which was initially undertaken to study another problem. This experiment had to be terminated because the corn silage fed in the ration became unfit for use; the opportunity was then taken to obtain additional information, even though limited, on the soybean and the soybean oil meal rations. Analysis of the data obtained reveals that the lambs responded somewhat more poorly to each ration than the lambs in the two experiments reported in detail in this paper. This was probably due to their being off feed for some time because of the poor quality of the corn silage previously fed. The results obtained for the two rations differ somewhat from those obtained in the other experiments as there was no difference in the digestibility of the nitrogen, and the soybean ration had an unexpectedly low average biological value, being 52 as compared with 62 for the soybean oil meal ration. The fact that unthrifty lambs responded differently from thrifty lambs when fed the soybean ration may possess some significance. However, because of the nature of the experiment, these data were not considered as reliable as the

other data herein reported and therefore were not considered in the summary of results.

DISCUSSION

All of the rations fed in these experiments seemed to be palatable to the lambs. Little difficulty was experienced in making abrupt changes from one ration to another in either of the experiments.

The data of experiment 1 and experiment 2 on the digestibility and the utilization of the nitrogen in the rations with ground soybeans, solvent-process soybean oil meal, and solvent-process meal with special heat treatment, have been combined and treated statistically for significance by the analysis of variance method (*17*). Treatment of these data in this fashion indicates certain highly significant differences.

The percentage of apparent digestibility of nitrogen for the two experiments averaged 62.9, 69.0, and 70.6 for the ground soybeans, solvent-process soybean oil meal, and the solvent-process soybean oil meal with special heat treatment, respectively. Statistical treatment of these data indicates that the difference in the digestibility of the nitrogen between the ration with the solvent-process meal and the ration with the toasted solvent-process meal was so small that it might well have been due to chance variation. However, each of the soybean oil meal rations was sufficiently higher in the digestibility of the nitrogen as compared with the soybean ration to give highly significant odds against such a difference being due to chance.

It is of interest to note that the lambs digested the nitrogen of the two solvent-process soybean oil meal rations with about the same efficiency as reported earlier by this station for an expeller-process soybean oil meal ration (*8*). In the earlier experiment, which involved four lambs, an average apparent digestibility of 71 percent was found for the nitrogen in a ration similar to those fed in this experiment, except that it contained expeller-process soybean oil meal. These data indicate that solvent-process soybean oil meal and expeller-process soybean oil meal have approximately the same apparent digestibility of nitrogen for lambs.

The percentage of total nitrogen stored for the two experiments averaged 18.2, 23.7, and 26.3, for the soybean ration, solvent meal ration, and the heat-treated meal ration, respectively. Again, statistical treatment indicates no significant difference between the two soybean oil meal rations, but each ration permitted a significantly greater storage of the total nitrogen than the soybean ration.

The differences in the percentage of digested nitrogen stored were not so consistent between rations as were the differences in the percentage storage of total nitrogen. The percentage storage of digested nitrogen gave average values of 28.5, 34.1, and 37.2 for the soybean ration, the solvent soybean oil meal ration, and the heat-treated soybean oil meal ration, respectively. Analysis of variance shows that the difference between the value for the soybean ration and either of the soybean oil meal rations was significant at the 5-percent level but not at the 1-percent level.

The biological values averaged 61, 62, and 63, respectively, for the soybean ration, the solvent meal ration, and the heat-treated meal

ration. Statistical treatment showed no significant differences in the biological values.

These results indicate that for growing lambs, the most significant difference between raw soybean protein and solvent-process soybean oil meal protein is in digestibility. The raw soybean protein was about 91 percent as digestible as the protein in the meals. Further heat treatment of the solvent-process meal resulted in little or no improvement in digestibility of the nitrogen by the lambs.

These data indicate a possible difference in the ability of the lambs to utilize the digested nitrogen of the raw soybean as compared with that of the solvent process meals. However, there was no significant difference between the three rations in the biological values. Therefore, while the lambs utilized the digested raw soybean nitrogen somewhat less efficiently than the digested nitrogen of the solvent-process meal, further work would be desirable before definite conclusions were drawn that this was a significant difference.

The idigestibility and utilization of the nitrogen in soybean flakes were s milar to the values for the raw soybeans, though slightly higher. The small differences were not statistically significant. Apparently, the amount of heat involved in flaking was insufficient to affect the protein to any appreciable degree.

The ration with toasted soybean oil meal and sufficient soybean oil to give the same fat content as the soybean ration had a slightly lower digestibility of nitrogen than the toasted soybean oil meal ration without added fat. The average values for the eight lambs were 71.1 percent and 73.1 percent, respectively. While this difference is small, in six out of the eight comparisons the high-fat ration was lower in digestibility of nitrogen than the low-fat ration. Statistical treatment by the analysis of variance method shows this difference to be somewhat too small for significance.

Since the experiments reported in this paper showed some differences in the digestibility of protein between rations, the question arose as to whether or not there might be differences in the digestibility of other nutrients. A complete digestibility study was not made of the various nutrients as these experiments were conducted solely to study the protein. However, since the air-dry weight of feces produced by the lambs on the different rations varied considerably, differences in the digestibility of the dry matter undoubtedly had occurred.

The dry-matter content of the feces was not determined as a part of these experiments and not all feces samples were still available when the data were summarized. In another experiment, the moisture content of 28 feces samples prepared for analysis in the same manner had an average dry-matter content of 92.5 percent. Using this average value, the digestibility of the dry matter was calculated for each ration in the two experiments reported in this paper.

In experiment 1, the average digestibility of the dry matter was 64 percent for the soybean ration, 66 percent for the soybean flake ration, 70 percent for the solvent-process soybean oil meal ration, and 70 percent for the toasted soybean oil meal ration. In experiment 2, the average values were 65 percent for the soybean ration, 69 percent for the solvent-process soybean oil meal ration, 70 percent for the toasted soybean oil meal ration, and 66 percent for the toasted soybean oil meal ration with added fat.

These data indicate that the digestibility of the dry matter was lower by the lambs when fed the soybean and the soybean flake rations than when fed either of the soybean oil meal rations. Further heat treatment of the solvent-process soybean oil meal did not affect the digestibility of the dry matter. The high-fat toasted soybean oil meal ration was significantly less digestible than the toasted soybean oil meal ration without the additional fat and of nearly the same digestibility as the soybean and soybean flake rations. These results indicate that the fat content of the soybeans and soybean flakes was largely responsible for the lower digestibility of the dry matter of these rations as compared with the soybean oil meal rations.

The effect of fat content on the digestibility of the dry matter was apparently more marked than on the digestibility of the protein alone, since the digestibility of the protein in the toasted meal, high-fat ration was slightly, but not significantly, lower than that of the toasted meal ration without the additional fat. Therefore, the higher digestibility of the soybean oil meal protein as compared with raw soybean protein was probably due both to the heat treatment and to the removal of the oil, but more largely to the heat treatment.

The presence or absence of soybean oil seemed to have little effect on the use lambs made of the digested soybean protein. In experiment 2, the soybean oil meal ration with added fat furnished nitrogen at least equal in efficiency to that of a similar soybean oil meal ration without the additional fat.

These data with lambs are similar to most of the data obtained with rats in showing that heat treatment improves the digestibility of soybean protein. However, these data differ in regard to the effect of heat treatment on the efficiency of the protein. Lambs do not utilize the heated protein with much, if any, more efficiency than raw soybean protein, whereas rats utilize the heated protein with considerably more efficiency.

Nitrogen-balance data at this station and elsewhere have shown that ruminants are less specific in their requirements for the commonly accepted essential amino acids than nonruminants. This has led to the belief that bacteria in the rumen synthesize nutritively complete protein from less complete feed protein, or from simple forms of nitrogen, and eventually this bacterial protein is available to the animal.

Before drawing practical applications from these experiments, it should be borne in mind that the soybean products furnished nearly all the protein in the experimental rations. Therefore, any differences between these sources of protein were magnified as compared with differences that might be expected in practical feed lot rations. For example, in lamb-feeding experiments now in progress at this station to compare ground soybeans and soybean oil meal, as well as other protein-rich feeds, as protein supplements to a basal ration of corn, corn silage, and minerals, the supplements furnished about 26 percent of the total protein in the rations. In similar feeding experiments with yearling steers, the supplements furnished approximately 30 percent of the total protein. Feed lot experiments in which the overall effects of rations are measured and in which protein is fed at fairly liberal levels may not bring out even fairly large differences in digestibility or utilization of the protein in the protein supplement.

SUMMARY

Nitrogen-balance experiments with lambs were conducted to determine the effect of heat treatment and oil extraction on the digestibility and utilization of soybean protein. The soybean feeds used in the experiments were raw soybeans, unextracted soybean flakes, solvent-process soybean oil meal, and heat-treated solvent-process soybean oil meal.

The rations, as fed, contained approximately 11 percent total protein on an air-dry basis, 10 percent being furnished by one of the soybean feeds and 1 percent by the low-protein basal ration. All nitrogen balances were of 10 days' duration, and the eight lambs in each experiment were fed each of the four rations tested in that particular experiment.

The apparent digestibility of protein for two experiments averaged 62.9 percent for the raw soybean ration, 69.0 percent for the solvent-process soybean oil meal ration, and 70.6 percent for the heat-treated solvent-process soybean oil meal ration. In a single experiment the protein in the soybean flake ration had a digestibility of 63.3 percent. The protein in a heat-treated soybean-oil meal ration with enough added soybean oil to equal that contained in the soybean ration had an average digestibility of 71.1 percent, as compared with an average of 73.1 percent for a similar ration without the added soybean oil, and 64.3 percent for the soybean ration.

The protein furnished by raw soybeans or unextracted soybean flakes had a significantly lower digestibility for lambs than the protein furnished by solvent-process soybean oil meal with or without special heat treatment. Apparently, this difference in digestibility was due mainly to the heat treatment given the meals since the addition of soybean oil did not significantly lower the digestibility of the protein.

The storage of total nitrogen intake in two experiments averaged 18.2 percent for the soybean ration, 23.7 percent for the solvent-process soybean oil meal ration, and 26.3 percent for the heat-treated solvent process soybean oil meal. In one experiment, the total nitrogen stored averaged 16.3 percent for the soybean flake ration as compared with 15.0 percent for the soybean ration. The storage of total nitrogen intake averaged 29.9 percent for the heat-treated soybean oil meal and 31.6 percent for a similar ration but with added soybean oil. The lambs stored significantly less of the total nitrogen intake when fed the soybean or the soybean flake ration than when fed any of the other rations. Further heat treatment of the solvent-process soybean oil meal did not result in a significant improvement as measured by storage of total nitrogen. The storage of total nitrogen was fully as high by the lambs when fed the soybean oil meal ration with added fat as when fed a similar ration without the added fat, and the storage was significantly higher than when the lambs were fed the soybean ration.

The difference in percentage of *total* nitrogen stored between the soybean or soybean flake ration and either of the solvent-process soybean oil meal rations was due chiefly to a lower digestibility. This was indicated by the fact that the differences in percentage of *digested* nitrogen stored between these rations were relatively smaller. The differences in biological values were so slight that they were not significant at all.

Contrary to data obtained with nonruminants, these data with lambs show that additional heat treatment of solvent-process soybean oil meal results in no appreciable improvement in the protein. The higher value of soybean oil meal protein, as compared with raw soybean protein, resulted mainly from higher digestibility rather than from greater efficiency in the utilization of the digested nitrogen.

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INFLUENCE OF VARIETY, LOCATION, FERTILIZER, AND STORAGE ON THE ASCORBIC ACID CONTENT OF POTATOES GROWN IN NEW YORK STATE [1]

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INTRODUCTION

In connection with a long-time research program designed to improve the potato industry in New York State (*22*),[2] the ascorbic acid content of several varieties of potatoes (*Solanum tuberosum* L.) grown in different parts of the State was determined, and the effect of soil reaction, fertilizer, temperature, and period of storage on the ascorbic acid content of the raw potato was studied. Incidental to studies of the culinary quality of these potatoes, some determinations were made of the ascorbic acid content of cooked potatoes. Observations made during three consecutive seasons (1937 to 1939) are reported here.

REVIEW OF LITERATURE

The factors reported as influencing the ascorbic acid content of foods have been summarized by the American Medical Association (*1*). The observations on potatoes which have a bearing on the present investigation will be mentioned briefly.

Varietal differences in ascorbic acid content of potatoes have been reported by a number of investigators (*3, 4, 6, 8, 9, 16, 18, 23, 24*). Ijdo (*4*) found variations up to 60 percent between varieties of potatoes, but not more than 10 percent within a variety. Esselen, Lyons, and Fellers (*3*) stated that although they found varietal differences, in many cases the difference within a variety was greater than that between varieties.

Decreases of from 50 to 70 percent in ascorbic acid content of potatoes stored from 6 to 12 months have been reported (*3, 6, 12, 13, 15, 17, 21, 23, 24*). Mayfield et al. (*9*) observed that the loss of ascorbic acid was greater when potatoes were stored in a cool, damp cellar (37° to 46° F.) than when stored in a warm, dry cellar (55° to 60°). The initial loss may be very rapid, as indicated by the studies of Scheunert et al. (*15*) and Olliver (*10*).

Although the location in which the vegetable is grown and the season have been shown to affect the vitamin C content of some vegetables, few observations have been reported on potatoes. Thiessen (*21*) observed that the vitamin C content of Bliss Triumph potatoes was fairly constant for the crops for 4 different years, the potatoes

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grown on dry land containing slightly more vitamin C than those grown on irrigated land. Esselen, Lyons, and Fellers (*3*) tested several varieties of potatoes grown in different sections of the country, and found no significant differences in their ascorbic acid content. Wachholder and Nehring (*24*) concluded, however, that some factor associated with location is more important with respect to vitamin C than is either fertilizer or variety.

Evidence as to the effect of fertilizers on the vitamin C content of potatoes is conflicting. Illyuviev and Ulanova (*5*) reported that nitrogen and potassium fertilizers increase the vitamin C content of potatoes. Scheunert and Reschke (*14*), however, after studying the vitamin C content of vegetables, including potatoes fertilized with farmyard manure and with manure plus nitrogen, phosphorus, and potassium fertilizers, concluded that the differences between individual specimens of the same plant were greater than those between specimens differently fertilized.

Ott (*11*) studied potatoes from field experiments in which the soils had been fertilized with mineral fertilizers containing various combinations of nitrogen, potassium, phosphorus, and calcium, with and without the addition of manure. He reported that fertilizing with nitrogen and phosphorus increased the vitmain C content of potatoes whereas potassium and calcium decreased it. The potatoes used in his experiments, however, had been kept at room temperature from the time of harvest, in October, until December before they were tested. At that time the ascorbic acid values ranged from 1.7 to 3.9 mg. per 100 gm. These low values suggest that storage had influenced the ascorbic acid content much more than fertilizer.

Wachholder and Nehring (*23*) found that ascorbic acid content was often higher when a mineral fertilizer was used than when stable manure was employed; however, as a result of later experiments (*24*), these authors concluded that fertilizer was of less importance than was location in its effect on the vitamin C content of potatoes.

Lyons and Fellers (*7*) found that the addition of certain minor elements (lead, boron, zinc, manganese, magnesium, mercury, and cobalt) to soils of varying acidity had little or no effect on the ascorbic acid content of potatoes grown on soils to which a complete fertilizer had been added.

EXPERIMENTAL PROCEDURES

The potatoes used in this study were supplied by the Department of Vegetable Crops of the New York State College of Agriculture. All stocks were practically disease-free.

Eight potatoes from each lot in the 1938 and 1939 crops were analyzed; in 1937 only four from each lot were tested.

STORAGE

When potatoes grown in various parts of the State were received in Ithaca, they were put into storage at 40° F. unless the effect of other storage temperatures was being investigated. A variable number of days elapsed between harvest and storage of the potatoes grown in the different localities, but this period at uncontrolled temperature was approximately the same for all varieties raised in a given locality in one year. All potatoes were tested within 2 days after they were

taken out of storage. Between time of removal from storage and analysis, they were kept in a refrigerator at a temperature of 40°. Since the period of storage influences the vitamin C content, the lots of potatoes to be compared were either analyzed during a relatively short time, or potatoes were tested from the several lots in rotation, two at a time, until the testing was finished.

<div align="center">SAMPLING</div>

After preliminary experiments with samples of cortex and medulla, lengthwise half slices and crosswise half slices, a crosswise center half slice from the pared potato was chosen for analysis. The paring, about one-sixteenth inch thick, was removed by means of a slit vegetable knife, and a 10-gm. sample was cut with a stainless-steel paring knife. The samples were immediately covered with the acid extracting medium to minimize opportunity for oxidation. Since the completion of this study, Rolf (*12*) has reported that the relative distribution of ascorbic acid between stem and bud end differed in Green Mountain, Irish Cobbler, and immature Chippewa potatoes, and was subject to continuous change throughout storage. Rolf's graphs showing these changes during storage, however, indicate that changes in the ascorbic acid content of the whole tuber, as indicated by an average of values obtained from stem end, bud end, and side pieces, is reflected in a change in ascorbic acid content of the side pieces. These latter correspond approximately to the center half slices chosen as the samples in this study.

Determinations on such samples from 52 old and 24 new potatoes showed, as Olliver (*10*), Rolf (*12*), and Esselen, Lyons, and Fellers (*3*) have reported, that there was no relation between ascorbic acid content of potatoes and tuber size. Hence no attempt was made to select potatoes of uniform size for analysis.

<div align="center">COOKING</div>

About 1 kg. of potatoes was washed, dried, and pared with a slit paring knife which gave a fairly uniform paring, about one-sixteenth inch thick. The pared potatoes were weighed, washed in a quart of cold water for about 30 seconds, and drained while a thermometer was being inserted into one potato of the lot. The potatoes were then put into a 5-quart enameled pan containing actively boiling, unsalted water. Two grams of water for each gram of unpared potato had been weighed before heating. The potatoes were cooked, covered, to an internal temperature of 97.8° C. (208° F.). After draining, the entire lot of cooked potatoes was mashed with an ordinary wire potato masher. Vitamin C determinations were made on 10-gm. samples in a manner similar to that described for the raw potatoes, except that it was unnecessary to use sand in grinding.

<div align="center">DETERMINATION OF ASCORBIC ACID</div>

Reduced ascorbic acid was determined by a modification of the method of Bessey and King (*2*). After preliminary tests with several acid solutions, a 2-percent solution of sulfuric acid, to which 2 percent of metaphosphoric acid was added, was chosen as the extracting medium. Values for blanks were always low, and there was no indication that sulfuric acid was unsuitable for this use, as suggested by the American Medical Association (*1*).

The samples were ground in a glass mortar .with acid-washed sand
and portions of the extracting medium.   Three extractions were made,
with approximately 30 ml. of acid solution each time; the material
obtained was centrifuged, and the combined extracts made up to vol-
ume in a 100-ml. flask.

Aliquot portions were titrated against a solution of sodium 2, 6-
dichlorobenzenoneindophenol which was standardized against a
solution of synthetic ascorbic acid.   Reduced ascorbic acid only was
determined, since preliminary tests showed no marked difference
between extracts treated with hydrogen sulfide and untreated extracts;
hence, the amount of ascorbic acid in the reversibly oxidized form
was considered to be negligible.

RESULTS AND DISCUSSION

EFFECT OF VARIETY ON ASCORBIC ACID CONTENT OF RAW POTATOES

Material for this part of the study was taken from replicated
variety-yield tests.   The seed was from the same source and the
potatoes had previously been stored under identical conditions.

Seven varieties of potatoes—Irish Cobbler, Chippewa, Earlaine,
Green Mountain, Houma, Katahdin, and Warba—grown in different
locations in the State in the seasons of 1937, 1938, and 1939, were
analyzed for vitamin C.   All of these varieties, except Warba, were
grown in Tompkins and Suffolk Counties in all three seasons; all
varieties were grown in Wayne County in the seasons of 1937 and 1938,
and Warba was grown in Tompkins County in these two seasons only.
All varieties were grown in Genesee County in 1939.   In addition, the
following varieties were tested: Sebago, grown in Tompkins and
Suffolk Counties in 1938 and 1939, in Wayne County in 1938 only,
and in Genesee County in 1939 only; Pioneer Rural, grown in Wayne
and Tompkins Counties in 1938, and in Genesee County in 1939;
Russet Rural, grown in Wayne and Tompkins Counties in 1938, and
in Genesee and Tompkins Counties in 1939; and Mohawk (U. S.
Department of Agriculture Seedling 46000), grown in Suffolk County
in 1938 and 1939.

With the exception of Katahdin, all varieties grown in different
locations in the three seasons were mature when harvested.   In 1937
and 1938, tubers of the variety Katahdin were nearly mature.   In
these years no records were kept of the dates of maturity, and early-
maturing varieties remained in the ground for some time before har-
vest.   In 1939, in Tompkins and Genesee Counties, the several
varieties were harvested soon after they matured, and were analyzed
for ascorbic acid content within a few days after harvest (table 1).
Of the other varieties tested, all were mature except Russet Rural and
Pioneer Rural, grown in Tompkins County in 1938, which were nearly
mature when harvested, and the potatoes of the variety Sebago, grown
in Tompkins County in 1938, which were immature.

Information concerning the soil in which the potatoes were grown
and the fertilizer treatment used in each location is given in the foot-
notes to table 1.   Although it is convenient to indicate the location
by the name of the county, it should not be inferred that the potatoes
grown on a given farm are representative of all potatoes grown in the
county.

TABLE 1.—*Mean ascorbic acid values for varieties of potatoes grown in different locations, during 3 seasons* [1]

Location and year	Date of harvest	Period of testing (after harvest)	Mean ascorbic acid value for—								
			Katahdin	Irish Cobbler	Chippewa	Earlaine	Warba	Houma	Green Mountain	Sebago	All varieties
		Days	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Tompkins:											
1937 [2]	Oct. 26	86–103	13.2	13.8	11.8	13.3	11.6	15.3	14.9	-----	13.2
1938 [3]	Sept. 28	13–50	29.3	19.5	20.3	25.0	20.8	25.5	21.6	19.3	22.6
1939 [3]	{Sept. 8, Oct. 3, Nov. 1}	3–7	28.1	40.3	29.6	45.3	-----	40.9	19.9	26.6	32.9
Suffolk:											
1937 [4]	Sept. 22	59–77	16.3	10.8	7.4	9.0	10.1	15.3	15.0	-----	11.4
1938 [4]	Sept. 5	29–71	14.8	12.0	8.6	14.5	10.3	13.8	11.0	12.3	12.2
1939 [4]	Sept. 5	13–15	27.9	21.3	22.8	24.5	-----	26.8	21.0	25.8	24.3
Wayne:											
1937 [5]	Sept. 1	119–125	13.4	5.8	8.7	6.1	7.5	11.8	9.9	-----	9.2
1938 [6]	Oct. 5	10–45	19.8	14.1	10.4	17.5	15.6	16.1	16.5	16.5	15.8
Genesee, 1939 [7]	{Sept. 8, Sept. 26}	3–8	36.6	30.3	22.3	33.4	-----	25.7	22.1	27.5	28.3

[1] For 1937 each mean for a variety within a county represents 4 tubers; for 1938 and 1939 each such mean represents 8 tubers. The values given are in milligrams per 100 gm. of raw potato.
[2] Grown on Dunkirk silt loam soil of heavy type; 1,000 pounds of 5–10–5 fertilizer added per acre.
[3] Grown on Lordstown stony silt loam soil; 1,000 pounds of 5–10–5 fertilizer added per acre.
[4] Grown on Sassafras silt loam soil; 2,000 pounds of 5–10–5 fertilizer added per acre.
[5] Grown on muck soil; 1,800 pounds of 4–8–12 fertilizer added per acre.
[6] Grown on muck soil; 2,250 pounds of 5–8–10 fertilizer added per acre.
[7] Grown on muck soil; 800 pounds of 8–16–16 fertilizer added per acre.

In 1937, the ascorbic acid determinations on these lots were made between December 17 and January 6; in 1938, between October 5 and November 29; and in 1939, between September 11 and November 7. The mean ascorbic acid values for a given variety varied from year to year, being lower when the storage period was longer. The mean ascorbic acid values for the varieties on which the most data were obtained are shown in table 1. The data were treated by analysis of variance, the data for each year being analyzed separately and the data for those varieties grown in the same locations in 1938 and 1939, together. Although considerable variation was observed between tubers of a given variety, for one location and year, there is a mathematically significant difference in vitamin C content between varieties, for the odds are greater than 99:1 against the chance occurrence of so great a difference.

The differences between means of certain varieties grown in three locations in 1938 and 1939 are given in table 2. Only those comparisons which seem most likely to represent real differences are shown. With few exceptions, differences between varieties being compared are in the same direction for both years and all locations. When data for varieties grown in Tompkins and Suffolk Counties in 1938 and 1939 were analyzed together, all the differences included in table 2 were significant at the 1-percent level. For these locations and years, the varieties Katahdin, Earlaine, and Houma tended to have high ascorbic acid values, whereas the ascorbic acid values for Chippewa and Green Mountain tended to be low, and those for Sebago and Cobbler, intermediate. Although the variety Warba was not grown in 1939, the most consistent differences between Warba and other varieties grown in 1938 are included in table 2. Mean values for Warba for 1937

TABLE 2.—Differences between means of ascorbic acid values of certain varieties of potatoes grown in 1938 and 1939 [1]

Comparison of varieties	Differences between means of varieties within a county						Differences between means of varieties, 3 locations, within a year		Differences between means of varieties for 2 years were analyzed together		
	Tompkins		Suffolk		Wayne	Genesee			Varieties grown in 1 year		Varieties grown in 2 counties in 2 years
	1938	1939	1938	1939	1938	1939	1938	1939	1938	1939	1938 and 1939
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Katahdin:											
Chippewa	−9.0**	[2] (+1.5)	−6.2**	−5.1	−9.4**	−14.3**	−8.2**	−6.0**	−7.6**	−1.8	−4.7**
Green Mountain	−7.7**	−8.2**	−3.8	−6.9	−3.3	−14.5**	−5.0**	−9.9**	−5.7**	−7.5**	−6.6**
Sebago	−10.0**	−1.5	−2.5	−2.1	−3.3	−9.1**	−5.3**	−4.3	−6.2**	−1.8	−4.0**
Warba	−8.5**		−4.5		−4.2		−5.8**				
Irish Cobbler:											
Earlaine	+5.5**	+5.0	+2.5	+3.2	+3.4	+3.1	+3.8**	+3.7	+4.0	+4.1	+4.0**
Houma	+6.0**	+.6	+1.8	+5.5	+2.0	[2] (−4.6)	+3.3**	+.4	+3.9	+3.0	+3.4**
Green Mountain	[2] (+2.1)	−20.4**	−1.0	−.3	[2] (+2.5)	[2] −8.2**	+1.1	−9.7**	+.5	−10.3**	−4.9**
Chippewa:											
Earlaine	+4.7	+15.7**	+5.9**	+1.7	+7.1**	+11.1**	+5.9**	+9.5**	+5.4*	+8.7**	+7.0**
Houma	+5.2	+11.3**	+5.2	+4.0	+5.7**	+3.4	+5.4**	+6.2**	+5.3**	+7.6**	+6.4**
Earlaine:											
Green Mountain	−3.4	−25.4*	−3.5	−3.5	−1.0	−11.3**	−2.7	−13.4**	−3.5	−14.4**	−8.9**
Sebago	−5.7**	−18.7**	−2.2	+1.3	−1.0	−5.9	−3.0	−7.8**	−4.0	−8.7**	−6.3**
Warba	−4.2		−4.2		−1.9		−3.5**				
Houma:											
Green Mountain	−3.9	−21.0**	−2.8	−5.8	+.4	+3.6	−2.2	−10.1**	−3.4	−13.3**	−8.3**
Sebago	−6.2**	−14.3**	−1.5	−1.0	[2] (+.4)	[2] (+1.8)	−2.5	−4.5	−3.9	−7.6**	−5.7**
Warba	−4.7		−3.5		−.5		−3.0				
Green Mountain:											
Sebago	[2] (−2.3)	+6.7	+1.3	+4.8	[2] (0)	+5.4	−.3	+5.6**	−.5	+5.7**	+2.6**
Least difference [3]	5.4**	8.1**	5.4**	8.1**	5.4**	8.1**	3.1**	4.7**	4.6**	4.6**	3.3**

[1] Each mean for a variety within a county represents 8 tubers; the values given are in milligrams per 100 gm. of raw potato.
[2] Values within parentheses are exceptions.
[3] Least difference between mean values required for odds of 99:1 against the chance occurrence of the difference.
** Significant at the 1-percent level.

(table 3) and 1938 (table 2) were consistently lower than those for Katahdin and Houma. When means of varieties for all locations within one year are compared, differences between Katahdin and Warba are significant at the 1-percent level for both 1937 and 1938, and differences between Houma and Warba are significant at the 1-percent level for 1937.

Since the potatoes were not grown, in all cases, in the same location within the county in 1937 as in 1938 and 1939, and since four tubers only from each lot were analyzed in 1937, the differences which appeared to be most significant for the 1937 crop are shown separately in table 3. In most instances the data obtained in 1937 support those

TABLE 3.—*Differences between means of ascorbic acid values of different varieties of potatoes grown in 3 locations in 1937* [1]

Comparison of varieties	Difference between means of varieties within a county			Difference between means of varieties in all counties
	Tompkins	Suffolk	Wayne	
Katahdin:	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Irish Cobbler	[2] (+0.6)	−5.5**	−7.6**	−3.9**
Chippewa	−1.4	−8.9**	−4.7**	−5.0**
Earlaine	[2] (+ .1)	−7.3**	−7.3**	−4.8**
Warba	−1.6	−6.2**	−5.9**	−4.5**
Irish Cobbler:				
Houma	+1.5	+4.5**	+6.0**	+3.7**
Green Mountain	+1.1	+4.2**	+4.1**	+2.9**
Chippewa:				
Houma	+3.5	+7.9**	+3.1	+4.8**
Green Mountain	+3.1	+7.6**	+1.2	+4.0**
Earlaine:				
Houma	+2.0	+6.3**	+5.7**	+4.6**
Green Mountain	+1.6	+6.0**	+3.8**	+3.8**
Warba:				
Houma	+3.7**	+5.2**	+4.3**	+4.3**
Green Mountain	+3.3	+4.9**	+2.4	+3.5**
Least difference [3]	3.7**	3.7**	3.7**	2.1**

[1] Each mean for a variety within a county represents 4 tubers; the values given are in milligrams per 100 gm. of raw potato.
[2] Values within parentheses are exceptions.
[3] Least difference between mean values required for odds of 99:1 against the chance occurrence of the difference.
**Significant at the 1-percent level.

obtained in 1938 and 1939. This is shown more clearly in table 4 in which the varietal means for all three locations together in each year are given, and their relative positions within the group are indicated. The ascorbic acid content of the varieties Katahdin and Houma was high in all years; that of Chippewa tended to be low; while that of Irish Cobbler, Warba, and Sebago was intermediate. Values obtained for Earlaine and Green Mountain were not consistent for the 3 years. These varieties may be more variable in ascorbic acid content, or since analyses were made earlier in each succeeding year, the observed differences might be related to different rates of loss of ascorbic acid during storage. Of the other varieties grown in one or two counties during 1938 and 1939, consistently low ascorbic acid values were found for Russet Rural potatoes grown in Wayne and Tompkins Counties in 1938, and in Tompkins and Genesee Counties in 1939; high ascorbic acid values were found for the variety Mohawk (U. S. Department of Agriculture Seedling 46000), grown in Suffolk County in both years; no consistent values were found for the variety Pioneer Rural.

TABLE 4.—*Mean of individual determinations for each variety grown in 3 locations in 1 year, and relative position in the group for each year* [1]

Variety	1937 [2]	Relative position	1938 [2]	Relative position	1939 [2]	Relative position
	Mg.		*Mg.*		*Mg.*	
Katahdin	14.3	1	21.3	1	30.9	3
Earlaine	9.5	5	19.0	2	34.4	1
Houma	14.1	2	18.5	3	31.1	2
Green Mountain	13.3	3	16.3	4	21.0	6
Sebago			16.0	[3] 4a	26.6	[3] 4a
Warba	9.8	[3] 4a	15.5	[3] 4b		
Irish Cobbler	10.4	4	15.2	5	30.7	4
Chippewa	9.3	6	13.1	6	24.9	5

[1] For years 1938 and 1939 each mean represents 24 potatoes, i. e., 8 tubers from each of 3 locations; for 1937 each mean represents 12 potatoes. The values given are in milligrams per 100 gm. of raw potato.
[2] Dates of analyses: 1937, Dec. 17 to Jan. 6; 1938, Oct. 5 to Nov. 29; 1939, Sept. 11 to Nov. 7.
[3] Since these varieties were grown for 2 years only, their relative positions are shown by a letter following a number.

No attempt was made to study the effect of leaving early and midseason varieties in the ground until the late varieties matured. It may be noted by inspection, however, that there was no apparent relation between the ascorbic acid content of a given variety and the season at which it matures. Among the late maturing varieties tested, ascorbic acid values for Katahdin were high, for Russet Rural, low, and for Sebago, intermediate. Among the midseason varieties tested, Houma had high and Chippewa, low, ascorbic acid values.

The varieties Irish Cobbler, Katahdin, Green Mountain, and Chippewa were among those tested by Esselen, Lyons, and Fellers (*3*). Of the five varieties grown in New York State which they tested, the ascorbic acid content of Katahdin potatoes was highest, 15.5 mg. per 100 gm., and that of Chippewa was lowest, 9.9 mg. per 100 gm. They found the mean ascorbic acid content of these varieties from several sources to be as follows: Irish Cobbler 13.1, Katahdin 12.5, Green Mountain 11.7, and Chippewa 9.7 mg. per 100 gm. These differences were not so striking as those found in the present experiment, in which the variety having the highest mean ascorbic acid value in a given year contained over 50 percent more than the variety having the lowest ascorbic acid value. Note that in 1939, when the tubers were analyzed soon after harvest, the mean values for all locations ranged from 21 to 34 mg. per 100 gm. for the several varieties. The mean value for all varieties and all locations for 1939 was approximately 29 mg. of ascorbic acid per 100 gm. If this value is taken as representative of potatoes at harvest, the ascorbic acid value of cooked potatoes soon after harvest would compare favorably with that of tomatoes, even if as much as 25 percent were lost in cooking.

In Tompkins County in 1939, three varieties had mean ascorbic acid values between 40 and 45 mg. per 100 gm. of raw potato. Many individual tubers had values between 40 and 50 mg., with one high value of 54 mg. per 100 gm. These values are comparable to the vitamin C content of citrus fruits.

EFFECT OF LOCATION ON THE ASCORBIC ACID CONTENT OF RAW POTATOES

The differences in the means of ascorbic acid content of potatoes grown in the several locations are shown in table 5. All differences were significant at the 1-percent level. The soils differed in the different locations, however, and in each location the usual amount of

fertilizer for the particular soil type was used. Hence differences in ascorbic acid content of potatoes grown in the several locations may have been due to soil type, fertilizer, or other local environmental conditions. Further studies are needed to determine which factors are involved.

EFFECT OF SOIL REACTION ON ASCORBIC ACID CONTENT OF RAW POTATOES

The potatoes used in this part of the study were grown in a long-time series of soil-reaction plots. The details of the experimental design and treatment of these plots have been published by Smith (*19, 20*).

Smooth Rural potatoes were grown in Tompkins County on soils of low, medium, and high reaction, with a pH of approximately 4.8, 6.8, and 8.0 or 8.1 in 1937 and 1938. The tubers were stored at 40° and 50° F. in 1937, and at 32°, 40°, and 50° in 1938. Since storage was one of the chief factors under investigation in 1937, four tubers from each lot were analyzed in October and November 1937, and in January, March, and May 1938.

The greatest differences in mean values for ascorbic acid in potatoes stored at different temperatures occurred in January; therefore, eight tubers from each lot grown in 1938 were analyzed in January 1939. The mean values for ascorbic acid content of these potatoes grown on soils of different reaction and stored at different temperatures are shown in tables 6 and 7.

TABLE 5.—*Differences between means of ascorbic acid values of potatoes grown in several locations* [1]

Comparison of locations (counties)	Separate analysis for each year			Analysis for 2 years together	
	[2] 1937	[3] 1938	[4] 1939	[4] 1938	[4] 1939
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Tompkins:					
Suffolk	−1.8**	−10.4**	−8.6**	−10.5**	−8.6**
Wayne	−4.0**	−6.8**			
Genesee			−4.6**		
Suffolk:					
Wayne	−2.2**	+3.6**			
Genesee			+4.0**		
Least difference [5]	1.4**	1.9**	3.1**	2.5**	2.5**

[1] Values given are in milligrams per 100 gm. of raw potato.
[2] Mean values for 28 potatoes were used in computing these differences.
[3] Mean values for 64 potatoes were used in computing these differences.
[4] Mean values for 56 potatoes were used in computing these differences.
[5] Least difference between mean values for odds of 99:1 against chance occurrence of the difference.
**Significant at the 1-percent level.

Differences between the means of ascorbic acid values of potatoes grown on soils of different reactions are shown in tables 8 and 9. The differences were not consistently in the same direction, and differences showing mathematical significance at the 1-percent level were found in two cases only, between tubers grown on soils of pH 6.8 and 8.1 and stored at 50° F. in March, for the 1937 crop, and between tubers grown on soils of pH 4.8 and 6.8 and stored at 40° in January, for the 1938 crop. These experiments afford no indication of an effect of soil reaction on the vitamin C content of potato tubers.

TABLE 6.—*Mean ascorbic acid values for Smooth Rural potatoes grown in 1937 on soils of different reaction and stored at 40° and 50° F.*[1]

Time of analysis and storage temperature (°F.),	Mean ascorbic acid value for potatoes grown on soil having a reaction (pH) of—			Average on all soils
	4.8	6.8	8.1	
October:	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Before storage	25.4	25.9	27.3	26.2
November:				
40	14.6	13.3	13.9	13.9
50	14.0	14.0	13.0	13.7
Average	14.3	13.7	13.5	13.8
January:				
40	9.5	8.7	7.4	8.5
50	12.1	11.6	13.1	12.3
Average	10.8	10.2	10.2	10.4
March:				
40	9.0	8.3	8.1	8.5
50	10.3	9.5	11.4	10.4
Average	9.7	8.9	9.8	9.4
May:				
40	7.6	7.8	7.6	7.7
50	8.6	7.9	8.2	8.2
Average	8.1	7.8	7.9	7.9

[1] Each mean for 1 soil reaction represents 4 potatoes; means for all reactions represent 12 potatoes. The values given are in milligrams per 100 gm. of raw potato.

TABLE 7.—*Mean ascorbic acid values for Smooth Rural potatoes grown in 1938 on soils of different reaction and stored at 32°, 40° and 50° F.*[1]

Storage temperature (°F.)	Mean ascorbic acid value for potatoes grown on soil having a reaction (pH) of—			Average on all soils
	4.8	6.8	8.0	
	Mg.	*Mg.*	*Mg.*	*Mg.*
32	8.1	[2] 9.9	7.7	8.5
40	11.5	8.7	9.3	9.8
50	15.0	14.5	13.9	14.5
Average	11.5	11.0	10.3	

[1] Each mean represents 8 potatoes. The values given are in milligrams per 100 gm. of raw potato. Analysis made in January 1939.
[2] Represents only 7 potatoes.

EFFECT OF STORAGE TEMPERATURE ON ASCORBIC ACID CONTENT OF RAW POTATOES

Smooth Rural potatoes lost approximately one-half of their original vitamin C content after a month of storage at 50° F. (table 10). The loss continued throughout the 7-month storage period at a decreased rate, until by the end of the period a total of 70 percent had been lost. Similar results were obtained at a storage temperature of 40°, except that the loss had reached approximately 70 percent by the end of the third month.

The differences between means of ascorbic acid values for potatoes stored at 40° and for those stored at 50° F. are shown in tables 11 and 12. Differences significant at the 1-percent level were found when tubers grown at all soil reactions and stored at 40° and 50° were com-

pared in January and March, for the 1937 crop, and in January, for the 1938 crop.   Differences between values for tubers stored at 32° and 50° (table 12) were also significant.   These studies indicate that losses of vitamin C when potatoes are stored at 50° tend to be less than losses when potatoes are stored at 40°.   Rolf (*12*) found that the vitamin C losses were less for potatoes stored at 15.5° C. (59° F.) than for those stored at 4.5° C. (40° F.).

TABLE 8.—*Differences between mean ascorbic acid values for Smooth Rural potatoes grown on soils of different reaction in 1937* [1]

Comparison according to soil pH	October, before storage	Time of analysis and storage temperature (° F.)											
		November			January			March			May		
		40	50	Average	40	50	Average	40	50	Average	50	50	Average
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
4.82:													
6.8	+0.5	−1.3	0.0	−0.6	−0.8	−0.5	−0.6	−0.7	−0.8	−0.8	+0.2	−0.7	−0.3
8.1	+1.9	−.7	−1.0	−.8	−2.1	+1.0	−.6	−.9	+1.1	+.1	0	−.4	−.2
6.8:													
8.1	+1.4	+.6	−1.0	−.2	−1.3	+1.5	0	−.2	+1.9**	+.9	−.2	+.3	+.1
Least difference [2]	3.1**				2.8**	2.8**		1.7**	1.7**	1.2**	1.6**	1.6**	

[1] Values given are in milligrams per 100 gm. of raw potato.
[2] Least difference between mean values for odds of 99:1 against chance occurrence of the difference.
**Significant at the 1-percent level.

TABLE 9.—*Differences between mean ascorbic acid values for Smooth Rural potatoes grown on soils of different reaction in 1938* [1]

Comparison according to soil pH	Storage temperature (°F.)			Average
	32	40	50	
4.8:	*Mg.*	*Mg.*	*Mg.*	*Mg.*
6.8	+1.8	−2.8**	−0.5	−0.5
8.0	−0.4	−2.2	−1.1	−1.2
6.8:				
8.0	−2.2	+0.6	−.6	−.7
Least difference [2]	2.6**	2.6**	2.6**	1.5**

[1] Each figure represents 8 potatoes. Values given are in milligrams per 100 gm. of raw potato; analyses made in January 1939.
[2] Least difference between mean values for odds of 99:1 against chance occurrence of the difference.
** Significant at the 1-percent level.

TABLE 10.—*Effect of storage and cooking on mean values for ascorbic acid content of Smooth Rural potatoes* [1]

Time of analysis	Ascorbic acid content at indicated storage temperature (°F.) of—					
	Raw potatoes [2]		Boiled potatoes [3]		Cooking Water [3]	
	40	50	40	50	40	50
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
October (before storage)	26.2	26.2	16.1	16.1	3.1	3.1
November	14.0	13.7	9.8	10.7	2.4	1.2
January	8.5	12.3	5.8	8.0	1.4	2.6
March	8.5	10.4	5.9	7.4	1.3	1.0
May	7.7	8.2	5.3	5.1	.7	.4

[1] Values given are in milligrams per 100 gm. of raw potato.
[2] Each figure represents 12 potatoes.
[3] Each figure represents 3 lots of cooked potatoes.

TABLE 6.—*Mean ascorbic acid values for Smooth Rural potatoes grown in 1937 on soils of different reaction and stored at 40° and 50° F.*[1]

Time of analysis and storage temperature (°F.),	Mean ascorbic acid value for potatoes grown on soil having a reaction (pH) of—			Average on all soils
	4.8	6.8	8.1	
October:	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Before storage	25.4	25.9	27.3	26.2
November:				
40	14.6	13.3	13.9	13.9
50	14.0	14.0	13.0	13.7
Average	14.3	13.7	13.5	13.8
January:				
40	9.5	8.7	7.4	8.5
50	12.1	11.6	13.1	12.3
Average	10.8	10.2	10.2	10.4
March:				
40	9.0	8.3	8.1	8.5
50	10.3	9.5	11.4	10.4
Average	9.7	8.9	9.8	9.4
May:				
40	7.6	7.8	7.6	7.7
50	8.6	7.9	8.2	8.2
Average	8.1	7.8	7.9	7.9

[1] Each mean for 1 soil reaction represents 4 potatoes; means for all reactions represent 12 potatoes. The values given are in milligrams per 100 gm. of raw potato.

TABLE 7.—*Mean ascorbic acid values for Smooth Rural potatoes grown in 1938 on soils of different reaction and stored at 32°, 40° and 50° F.*[1]

Storage temperature (°F.)	Mean ascorbic acid value for potatoes grown on soil having a reaction (pH) of—			Average on all soils
	4.8	6.8	8.0	
	Mg.	*Mg.*	*Mg.*	*Mg.*
32	8.1	[2] 9.9	7.7	8.5
40	11.5	8.7	9.3	9.8
50	15.0	14.5	13.9	14.5
Average	11.5	11.0	10.3	

[1] Each mean represents 8 potatoes. The values given are in milligrams per 100 gm. of raw potato. Analysis made in January 1939.
[2] Represents only 7 potatoes.

EFFECT OF STORAGE TEMPERATURE ON ASCORBIC ACID CONTENT OF RAW POTATOES

Smooth Rural potatoes lost approximately one-half of their original vitamin C content after a month of storage at 50° F. (table 10). The loss continued throughout the 7-month storage period at a decreased rate, until by the end of the period a total of 70 percent had been lost. Similar results were obtained at a storage temperature of 40°, except that the loss had reached approximately 70 percent by the end of the third month.

The differences between means of ascorbic acid values for potatoes stored at 40° and for those stored at 50° F. are shown in tables 11 and 12. Differences significant at the 1-percent level were found when tubers grown at all soil reactions and stored at 40° and 50° were com-

pared in January and March, for the 1937 crop, and in January, for the 1938 crop. Differences between values for tubers stored at 32° and 50° (table 12) were also significant. These studies indicate that losses of vitamin C when potatoes are stored at 50° tend to be less than losses when potatoes are stored at 40°. Rolf (*12*) found that the vitamin C losses were less for potatoes stored at 15.5° C. (59° F.) than for those stored at 4.5° C. (40° F.).

TABLE 8.—*Differences between mean ascorbic acid values for Smooth Rural potatoes grown on soils of different reaction in 1937* [1]

Comparison according to soil pH	October, before storage	Time of analysis and storage temperature (° F.)											
		November			January			March			May		
		40	50	Average	40	50	Average	40	50	Average	50	50	Average
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
4.82:													
6.8	+0.5	−1.3	0.0	−0.6	−0.8	−0.5	−0.6	−0.7	−0.8	−0.8	+0.2	−0.7	−0.3
8.1	+1.9	−.7	−1.0	−.8	−2.1	+1.0	−.6	−.9	+1.1	+.1	0	−.4	−.2
6.8:													
8.1	+1.4	+.6	−1.0	−.2	−1.3	+1.5	0	−.2	+1.9**	+.9	−.2	+.3	+.1
Least difference [2]	3.1**	------	------	------	2.8**	2.8**	------	1.7**	1.7**	1.2**	1.6**	1.6**	------

[1] Values given are in milligrams per 100 gm. of raw potato.
[2] Least difference between mean values for odds of 99:1 against chance occurrence of the difference.
**Significant at the 1-percent level.

TABLE 9.—*Differences between mean ascorbic acid values for Smooth Rural potatoes grown on soils of different reaction in 1938* [1]

Comparison according to soil pH	Storage temperature (°F.)			Average
	32	40	50	
	Mg.	*Mg.*	*Mg.*	*Mg.*
4.8:				
6.8	+1.8	−2.8**	−0.5	−0.5
8.0	−0.4	−2.2	−1.1	−1.2
6.8:				
8.0	−2.2	+0.6	−.6	−.7
Least difference [2]	2.6**	2.6**	2.6**	1.5**

[1] Each figure represents 8 potatoes. Values given are in milligrams per 100 gm. of raw potato; analyses made in January 1939.
[2] Least difference between mean values for odds of 99:1 against chance occurrence of the difference.
** Significant at the 1-percent level.

TABLE 10.—*Effect of storage and cooking on mean values for ascorbic acid content of Smooth Rural potatoes* [1]

Time of analysis	Ascorbic acid content at indicated storage temperature (°F.) of—					
	Raw potatoes [2]		Boiled potatoes [3]		Cooking Water [3]	
	40	50	40	50	40	50
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
October (before storage)	26.2	26.2	16.1	16.1	3.1	3.1
November	14.0	13.7	9.8	10.7	2.4	1.2
January	8.5	12.3	5.8	8.0	1.4	2.6
March	8.5	10.4	5.9	7.4	1.3	1.0
May	7.7	8.2	5.3	5.1	.7	.4

[1] Values given are in milligrams per 100 gm. of raw potato.
[2] Each figure represents 12 potatoes.
[3] Each figure represents 3 lots of cooked potatoes.

TABLE 11.—*Differences between means of ascorbic acid values of Smooth Rural potatoes grown in 1937 and stored at 40° and 50° F.*[1]

Time of analysis	Storage temperatures (° F.) compared	Soil reaction (pH)			
		4.8	6.8	8.1	Average
		Mg.	*Mg.*	*Mg.*	*Mg.*
November	40:				
	50	−0.6	+0.7	−0.9	−0.3
January	40:				
	50	+2.6	+2.9**	+5.7**	+3.8**
	Least difference [2]	2.8**	2.8**	2.8**	1.6**
March	40:				
	50	+1.3	+1.2	+3.3**	+1.9**
	Least difference [2]	1.7**	1.7**	1.7**	1.0**
May	40:				
	50	+1.0	+.1	+.6	+.5
	Least difference [2]	1.6**	1.6**	1.6**	.9**

[1] Means for each soil reaction, storage time, and temperature represent 4 tubers. Values given are in milligrams per 100 gm. of raw potato.
[2] Least difference between mean values for odds of 99:1 against chance accurrence of the difference.
**Significant at the 1-percent level.

TABLE 12.—*Differences between means of ascorbic acid values for Smooth Rural potatoes grown in 1938 and stored at different temperatures* [1]

Storage temperatures (° F.) compared	Soil reaction (pH)			
	4.8 [2]	6.8 [2]	8.0 [2]	Average [3]
	Mg.	*Mg.*	*Mg.*	*Mg.*
32:				
40	+3.4**	−1.2	+1.6	+1.3
50	+6.9**	+4.6**	+6.2**	+6.0**
40:				
50	+3.5**	+5.8**	+4.6**	+4.7**
Least difference [4]	2.6**	2.6**	2.6**	1.5**

[1] Analyzed in January 1939. Values given are in milligrams per 100 gm. of raw potato.
[2] Each figure represents 8 potatoes.
[3] Each figure represents 24 potatoes.
[4] Least difference between mean values for odds of 99:1 against chance occurrence of the difference.
**Significant at the 1-percent level.

EFFECT OF FERTILIZER TREATMENT ON THE ASCORBIC ACID CONTENT OF RAW POTATOES

VARIATIONS IN FERTILIZER ANALYSIS

Fertilizer containing nitrogen, phosphorus, and potassium in the ratio of 1–2–3 was varied by leaving out part or all of each of the three elements, one at a time. The equivalent of 2,000 pounds per acre of each of the fertilizers was added to the soil on which potatoes were grown. The potatoes used for the experiment were grown in Oswego County on muck soil. The variety Chippewa was used in 1938, and Green Mountain, in 1939. The average values obtained for the 2 years are shown in table 13; the difference between these values and the least difference neecssary to show mathematical significance at the 1-percent level appear in table 14.

TABLE 13.—*Effect of fertilizers of different composition on the ascorbic acid content of potatoes* [1]

Year	Analysis of fertilizer (N–K–P)						
	4–8–12	0–8–12	4–0–12	4–8–0	2–8–12	4–4–12	4–8–8
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1938 [2]	9.2	6.5	9.3	7.4	9.9	9.4	7.9
1939 [2]	13.3	12.6	12.0	14.3	12.0	13.4	13.6
Average [3]	11.4	9.6	10.7	10.9	10.7	11.6	10.7

[1] Values given are in milligrams per 100 gm. of raw potato.
[2] Each figure represents 8 potatoes.
[3] Each figure represents 16 potatoes.

TABLE 14.—*Differences between means of ascorbic acid values of potatoes treated with fertilizers having different analyses for N, P, and K* [1]

Variant	Analyses compared	Each year analyzed separately		Two years analyzed together [3]
		1938 [2]	1939 [2]	
		Mg.	*Mg.*	*Mg.*
N	4–8–12 and 2–8–12	+0.7	−1.3	−0.7
	2–8–12 and 0–8–12	−3.4**	+.6	−1.1
	4–8–12 and 0–8–12	−2.7**	−.7	−1.8**
P	4–4–12 and 4–4–12	+.2	+.1	+.2
	4–4–12 and 4–0–12	−.1	−1.4	−.9
	4–8–12 and 4–0–12	+.1	−1.3	−.7
K	4–8–12 and 4–8–8	−1.3	+.3	−.7
	4–8–8 and 4–8–0	−.5	+.7	+.2
	4–8–12 and 4–8–0	−1.8	+1.0	−.5
Least difference, 1 year [4]		2.4**	1.9**	
Least difference, 2 years [4]		2.3**	2.3**	1.6**

[1] Values given are in milligrams per 100 gm. of raw potato.
[2] Mean values for 8 potatoes were used in computing these differences.
[3] Mean values for 16 potatoes were used in computing these differences.
[4] Least difference between mean values for odds of 99:1 against chance occurrence of the difference.
**Significant at the 1-percent level.

None of the variations in phosphorus or potassium produced differences which were significant at the 1-percent level. Omission of nitrogen from the fertilizer was accompanied by a mathematically significant decrease in the ascorbic acid content of the potatoes grown in 1938, but not in those grown in 1939. Possibly the nitrogen applied in 1939 was less available than that applied in 1938 because of the difference in rainfall in the two seasons. The United States Weather Bureau station in the city of Oswego recorded for 1938 15.39 inches of rainfall from May through September and 8.10 inches for June, July, and August, as compared with 8.07 inches from May through September and 4.67 inches for June, July, and August, 1939. No records of rainfall were made at the farm where the potatoes were grown.

MINOR ELEMENTS

Smooth Rural potatoes, grown in Steuben County on gravelly silt loam having 1,000 pounds per acre of 5–10–5 fertilizer, were treated as follows: To one lot no additions were made; to a second lot were added six minor elements, copper, zinc, boron, manganese, magnesium, and iron; to a third lot was added 12 tons per acre of barnyard manure. Eight potatoes of each lot were tested for ascorbic acid content.

The average ascorbic acid values were 11.3, 12.4, and 11.5 mg. per 100 gm. for the three lots. None of the differences are mathematically significant, even at the 5-percent level. In both this study and that of Lyons and Fellers (7), boron, zinc, manganese, and magnesium were used; but this series also included copper and iron, whereas that of Lyons and Fellers included lead, mercury, and cobalt. The ascorbic acid content of potatoes grown on soils to which a complete fertilizer was added was not affected in either case.

EFFECT OF BOILING ON ASCORBIC ACID CONTENT OF POTATOES

The results of the cooking tests made on stored potatoes in the season of 1937–38 are shown in table 10. On an average, approximately two-thirds of the vitamin C value of the raw tuber remained in the boiled potato, and an additional amount was found in the cooking water. Actual destruction of ascorbic acid varied considerably, but was under 20 percent in 18 of 27 cooking tests.

SUMMARY

Varietal differences were observed in the ascorbic acid content of potatoes grown in different localities in New York State in three seasons. High values were obtained for the varieties Katahdin and Houma in all 3 years; the values for Chippewa tended to be low; while those for Irish Cobbler, Warba, and Sebago were intermediate. Less consistent results were obtained for Earlaine and Green Mountain.

The ascorbic acid content of potatoes grown in different locations showed marked variation. Whether this was due to fertilizer, soil type, climate, or other conditions is not known.

Under the conditions of these experiments neither soil reaction, the amount of nitrogen, phosphorus, and potassium in the fertilizer, nor the addition of minor elements to soil to which a complete fertilizer had been added had a consistent influence on the ascorbic acid content of potatoes.

Losses of ascorbic acid tended to occur more slowly in potatoes which were stored at 50° F. than in those stored at 40°.

Under the conditions of these experiments approximately two-thirds of the ascorbic acid content of the raw tuber remained in the boiled potato.

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SELF-INCOMPATIBILITY IN SEVERAL SPECIES OF RIBES IN THE WESTERN STATES [2]

By H. R. OFFORD, *pathologist*, and CLARENCE R. QUICK and VIRGIL D. MOSS, *associate forest ecologists, Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture* [4]

INTRODUCTION

The quantity and viability of seeds produced by *Ribes* [5] in the white pine areas of the western part of the United States are important factors in the control of the destructive blister rust disease of white pines (*Pinus* spp.) caused by the fungus *Cronartium ribicola* Fischer. Primary control is accomplished by suppressing the populations of *Ribes* within stands of white pine to the point where they cannot cause significant damage (*2*). Thereafter the factors affecting regeneration of the species from this residuum are of special interest because of their bearing upon the planning of long-range control.

Plant competition, shade, drought, winter-kill, physical injury, fungi, and insects are recognized as the principal factors unfavorable to the regeneration of *Ribes*. Reestablishment of *Ribes* in white pine forests can be discouraged by proper forest management based on correct interpretation of the ecology of pine and *Ribes* (*1*). Of the many factors involved in the reestablishment of *Ribes* in a control area this paper is primarily concerned with one, seed production.

The flowers of *Ribes* are perfect and homomorphic. Under natural conditions in forested areas fruiting is generally heaviest when the plants are gregarious, that is, when an abundance of pollen is close at hand. Insects are the chief carriers of *Ribes* pollen, though wind and water likewise disseminate it. The annual output of *Ribes* seeds depends on the weather at the time of flowering, the activity of insect pollinators, and the consumption of fruit and seeds by rodents. *Ribes* fruits are highly favored by mice, chipmunks, and ground squirrels, which eat and destroy the seeds. One caged chipmunk, [6] overnight, ate and destroyed about 6,000 seeds of *R. viscosissimum* Pursh. Birds scatter *Ribes* seeds but apparently do not necessarily destroy

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their viability. C. R. Quick collected droppings from robins and chickadees and subsequently obtained 60 to 90 percent germination of recovered seeds of *R. cereum* Dougl. and *R. nevadense* Kell. The bird droppings probably included both regurgitated and fecal material.

PREVIOUS WORK

Little work seems to have been done on controlled pollination of the native wild *Ribes* of the United States. A posthumous publication by East (*4, p. 472*) stated that "In the Saxifragaceae * * * there is much homogamy, though there are certain highly protandrous and protogynous species," and that many tested species of *Deutzia*, *Hydrangea*, *Philadelphus*, and *Ribes* appeared to be self-fertile. However, according to Karl Sax,[7] who prepared East's paper for publication, this statement was probably based on general information and observation and not upon specific data; it may have referred to varieties of cultivated currants and gooseberries. Janczewski (*6, p. 220*), although not commenting specifically on compatibility in *Ribes*, made the following statement which may indicate that he believed the plants to be sometimes self-fertile:

> The flowers of *Ribes* are frequently visited and pollinated by insects. The homogamous flowers may well be passed by, for their pollen easily falls on the stigmata ready to receive it. [Translated from the French.]

To obtain further information on fruit production in *Ribes*, experiments were undertaken to answer the question, Do certain *Ribes*, native to the white pine areas of the Western States, produce viable seed equally well by cross-pollination and self-pollination, or is one type of pollination chiefly responsible for setting the fruits?

METHODS AND MATERIALS

POLLINATION

In March 1940 hand-pollination of *Ribes* flowers (within species) was undertaken on *R. glutinosum* Benth., *R. roezlii* Regel, and *R. gracillimum* Cov. and Britt., which had been transplanted to the Botanical Garden of the University of California at Berkeley.[8] In May of the same year the work was extended to *R. roezlii* and *R. nevadense* growing naturally in the Sierra Nevada of California, and to *R. viscosissimum* and *R. lacustre* (Pers.) Poir. in northern Idaho. In May 1941 tests were repeated on *R. roezlii* in the Sierra Nevada. The period of flowering for *Ribes* is short, usually not more than a month under favorable conditions. For *R. roezlii*, in particular, the schedules for controlled pollination were such that any cyclic changes in compatibility should have been reflected in the results.

All pollination work was carried out by the usual emasculation and bagging procedure. Half-pound kraft bags were used to exclude unwanted pollen from flowers and inflorescences. Implements were kept sterile by frequent immersion in ethyl alcohol. The technique employed is believed to have met fully the requirements that (1) the pollen should be good, (2) the stigma should be receptive, and (3) the stigma should receive an adequate supply of pollen.

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[7] Correspondence.
[8] In cooperation with the Botanical Garden, directed by T. H. Goodspeed, the Bureau of Entomology and Plant Quarantine maintains a collection of about 80 species of *Ribes*.

Since flowers of the currants are relatively small, an inflorescence rather than a single flower was taken as the working unit. Thus the several flowers of a raceme were usually emasculated and enclosed within the same protective bag. For convenience the gooseberries were handled in a similar manner, although they had only one to three flowers per raceme, and several closely approximate flowers were often enclosed in one bag.

Since the *Ribes* flowers involved in these tests, especially *R. roezlii*, tended to be protogynous, care was taken to select flower buds from which the pistil did not protrude. The protogynous character of these flowers suggests that cross-pollination would be favored under natural conditions.

In many cases reciprocal crosses were made; that is, pollen from previously bagged selfs on one bush was used on the bagged crosses of a second bush, and pollen from selfs on the second bush was used on crosses of the first bush. In the remaining cases pollen for crosses came from plants not otherwise involved. All pollen from unbagged flowers used in the experiments was taken from unopened buds and from plants reasonably remote from those on which it was used. The chance that pollen from clones was involved is therefore small.

The selfs were pollinated by anthers from within the same protective bag. After being bagged, both the selfs and the crosses were pollinated two or three times at intervals of 2 to 4 days. With a few exceptions each bush involved in the experiment carried an equal number of bags for self-pollination and cross-pollination.

SEED GERMINATION

Ripe fruits were collected during the course of field and garden tests to permit a comparison of the viability of seed from natural and controlled sources.

The open-pollinated seeds of *Ribes glutinosum* and *R. nevadense*, and of *R. roezlii* from the Sierra Nevada, were collected from the same bushes on which the pollination work was done; those of *R. viscosissimum* from northern Idaho and of *R. roezlii* from the University of California Botanical Garden were taken from other bushes in the same locality.

The seeds that matured from the several controlled-pollination tests and those obtained from natural pollination were extracted (7) from the fruits during the fall of 1940 and on December 3 were planted in autoclaved river sand wet with Hoagland's (5) mineral nutrient solution. The seeds of each species were planted in flats of wet sand, treated with copper oxalate at the rate of 6 gm. per square foot of culture surface to control damping-off (8), and then stratified for a period of time and at a temperature that previous experience had shown was most satisfactory.

The first, or primary, stratification treatments were as follows: *Ribes glutinosum*, at 2.2° C. for 20 weeks; *R. nevadense*, at 0° for 24 weeks; *R. roezlii*, at 2.2° for 18 weeks; and *R. viscosissimum*, at 0° for 24 weeks. The second, or retrial, stratification treatments for all the species were generally at 0° for 20 weeks. Germination periods in the greenhouse were of 5 weeks' duration.

RESULTS OF TESTS

The tests on *Ribes gracillimum* were unsuccessful because of a serious twig blight, which damaged the plants and prevented setting of fruits by either cross- or self-pollination. The protective bags on *R. lacustre* were disturbed by some person or animal; therefore, the tests could not be considered as valid. In the other species ripe fruits were obtained in varying amounts from cross-pollination, but no fruits were formed as a result of self-pollination. Table 1 reports the results of the pollination tests.

No difficulties were experienced in the germination tests on the collected seeds. As shown in table 2, viable seed was produced by the controlled cross-pollination work.

TABLE 1.—*Comparison of number of fruits and seeds from controlled cross- and self-pollination of several* Ribes *species native to the Western States*

Ribes species	Location of test plants	Cross-pollination tests					Self-pollination tests		
		Inflo-res-cences	Flow-ers	Fruits set	Ma-tured fruits	Seeds	Inflo-res-cences	Flow-ers	Ma-tured fruits
		No.	No.	No.	No.	No.	No.	No.	No.
	University of California Botanical Garden, Berkeley.	8	[1] 32	11	10	496	7	[1] 28	0
R. roezlii	Sierra National Forest, Chowchilla Mountain.	25	144	65	[2] 35	1,737	25	160	0
	Stanislaus National Forest, Cow Creek.	51	217	116	[2] 56	([3])	49	258	0
R. glutinosum	University of California Botanical Garden, Berkeley.	12	84	34	30	1,010	12	[1] 72	0
R. nevadense	Sierra National Forest, Chowchilla Mountain.	16	80	39	32	725	16	83	0
R. viscosissimum	Clearwater Timber Protective Association, Idaho, Deer Creek and Powder House.	14	64	21	13	376	30	135	0
Total		126	621	286	176	4,344	139	736	0

[1] Number of flowers estimated from average number per bag proposed for the tests.
[2] Some additional fruits were destroyed by rodents.
[3] Seeds were not extracted.

TABLE 2.—*Comparison of data for germination tests on* Ribes *seeds from controlled cross- and open-pollinated flowers, 1940*

Ribes species	Location of test plants	Cross-pollinated seeds				Open-pollinated seeds			
		Cul-tures	Seeds	Seed-lings	Ger-mina-tion	Cul-tures	Seeds	Seed-lings	Ger-mina-tion
		No.	No.	No.	Per-cent	No.	No.	No.	Per-cent
R. roezlii	University of California Botanical Garden, Berkeley.	6	496	444	89.5	5	[1] 500	451	90.2
	Sierra National Forest	20	1,435	817	56.9	5	452	408	90.3
R. glutinosum	University of California Botanical Garden, Berkeley.	15	939	876	93.3	9	900	798	88.7
R. nevadense	Sierra National Forest	15	724	567	78.3	4	400	299	74.8
R. viscosissimum	Clearwater Timber Protective Association, Idaho.	8	376	284	75.5	3	[1] 300	187	62.3
Total or average.		64	3,970	2,988	75.3	26	2,552	2,143	84.0

[1] Seeds from same locality as cross-pollinated seeds, but not from same bushes.

DISCUSSION

Self-incompatibility in *Ribes roezlii*, *R. glutinosum*, *R. nevadense*, and *R. viscosissimum* is clearly shown by the data in table 1. From the 736 *Ribes* flowers that were self-pollinated, not a single mature fruit was obtained. On the other hand, cross-pollination of 621 flowers resulted in the setting of 286 fruits. From cross-pollination 176 mature fruits were ultimately obtained for seed-germination tests. More would have been obtained had not rodents destroyed a number of the experimental fruits about the time they were getting ripe. Other fruits were lost through natural dropping before they had matured.

Under natural conditions many *Ribes* flowers fail to produce fruits. The following records are a quantitative measure of this statement and show that the 46 percent of fruit set from controlled pollination is about what we might expect from a natural fruit crop.

At Cow Creek, Stanislaus National Forest, a study of fruiting habits was made on a series of *Ribes roezlii* bushes growing on a 1936 burn in mature timber and fruiting for the first time in 1941. Practically all the bushes originated in 1937, the year after the fire. On May 21, 1941, 37 bushes were bearing 974 flowers. Many more bushes of the same age did not flower or fruit in 1941. As the season progressed, the total number of fruits in various stages of maturity remaining on the 37 bushes were as follows: 376 on June 3, 109 on June 18, 42 on July 11, and 25 on August 13. The average stage of development of the 376 fruits present on June 3 was approximately the same as that of the 192 *R. roezlii* fruits set under controlled cross-pollination as reported in table 1. In another study in the spring of 1937, a favorable year for flower and fruit production, it was found that on 26 vigorous 5- to 6-year-old *R. viscosissimum* bushes occurring naturally in Upper Basin, St. Joe National Forest, Idaho, 427 racemes developed 3,847 flowers, which in turn produced 1,752 mature fruits. In the instances cited, 39 percent of the *R. roezlii* flowers and 45 percent of the *R. viscosissimum* flowers produced fruits under natural conditions.

East (3) and others have shown that when self-incompatibility is conditioned by a series of multiple allelomorphs the existence of cross-incompatible plants in the same population is a necessary corollary. The fact that in controlled crosses, as well as under natural conditions, less than 50 percent of the flowers set fruits agrees with the assumption that some degree of cross-incompatibility also exists in the *Ribes* species tested.

The reaction of *Ribes nevadense* to self-pollination was quite different from that of the other *Ribes* species. Of the 83 test flowers of *R. nevadense* 15 formed abortive fruits, whereas only 1 abortive fruit was set by each of the species *R. roezlii* and *R. viscosissimum*, and none by *R. glutinosum*.

Rodents took a heavy toll of *Ribes roezlii* fruits, but since the loss did not occur until fruits were well formed and nearly mature, it was not involved in the results shown in table 1 for the self-pollination tests.

Data in table 2 show that controlled cross-pollination as practiced in the tests of 1940 resulted in the formation of viable seed, and that, with one exception, the viability of the experimentally produced seed

compared favorably with that of seed resulting from open pollination. In the tests on *Ribes roezlii* from the Sierra National Forest, low viability was encountered in cross-pollinated seeds taken from 2 bushes, and average germination of only 2 percent was obtained in 6 of the 20 cultures. The remaining 14 cultures averaged 82 percent germination, which is comparable to that of the open-pollinated sample. In view of the variations that are possible in the results of greenhouse germination tests, and insofar as the interest of this report is concerned, no significance is attached to these differences. Observations and experimental data of the past 10 years have shown that the physical and physiological characteristics of *Ribes* seed vary considerably. With these limitations in mind it can be stated that the controlled pollination produced seeds that were normal in respect to average number per fruit, and in respect to size, color, weight, and germination.

It cannot be concluded from these tests that isolated bushes of *Ribes roezlii, R. nevadense, R. viscosissimum,* and *R. glutinosum* will never produce mature fruits under natural conditions, because little evidence is available on the distance of flight and the habits of insects in searching for and pollinating *Ribes* flowers, and because some degree of self-compatibility may be possible. The chances of fruit production, however, would appear to become progressively poorer as the number of *Ribes* bushes per acre is reduced by eradication work and by natural suppression. Furthermore, since rodents have a strong liking for fruits and seeds of *Ribes*, their consumption of fruit becomes more important when viewed in the light of a diminishing crop. These factors should aid the natural suppression of *Ribes* which normally takes place in ecologically maturing forest stands, and they should mean that the efforts of artificial suppression will be expedited by natural phenomena once the number of flowering *Ribes* per acre has been substantially reduced.

SUMMARY

In the spring of 1940 controlled pollination of 4 species of *Ribes* native to the Western States was undertaken. These tests were made in the Botanical Garden of the University of California at Berkeley on transplanted *R. roezlii* and *R. glutinosum*, in the Sierra Nevada of California on naturally occurring *R. roezlii* and *R. nevadense*, and in northern Idaho on naturally occurring *R. viscosissimum*. Tests were repeated in 1941 on *R. roezlii* occurring in the Sierra Nevada. Cross-pollination was successful for all the species tested, and produced 176 mature fruits from 621 flowers that were pollinated. Self-pollination of the same species under similar conditions failed to produce a single mature fruit. It is concluded, therefore, that under natural conditions the seed-bearing fruits of these 4 species of *Ribes* normally result from cross-pollination.

Viability of seed obtained by controlled cross-pollination in the tests of 1940 compared favorably with that of seed resulting from open pollination occurring naturally on the same plants of the same species, or on plants of the same species in the immediate locality. The average number of seeds per fruit and the percentage of flowers which set fruits were substantially the same for open and controlled pollination.

Self-incompatibility of *Ribes* will prevent heavy fruiting in scattered light populations of these plants. Also, rodent pressure is of increased significance when fruits are scarce. Together, these factors should simplify the control of blister rust disease in the white pine areas of the Far West.

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HUSK DEVELOPMENT OF SWEET CORN AS AFFECTED BY MOISTURE SUPPLY, AN IMPORTANT FACTOR IN CORN EARWORM CONTROL [1]

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INTRODUCTION

The treatment of ears of a given variety of sweet corn (*Zea mays L.*) grown in different fields during different seasons of the year with mineral oil containing insecticides for control of the corn earworm (*Heliothis armigera* (Hbn.)) has been found to vary in effectiveness. Such variation has been noticed even in fields planted from the same lot of seed and grown under conditions that seemed to be similar except as to time of planting. It is known that the effectiveness of this treatment, which is directed against the larvae after they have entered the ears and while they are concentrated in and feeding on the silk lying between the tip of the husk and the tip of the cob, is dependent on characters of the husk, including its extension beyond the tip of the cob. Therefore, it was thought that a comparison of husk measurements made at the time of roasting-ear harvest in fields of the same variety planted on different dates might give some explanation for these differences in control. Measurements were made of a large number of ears of many varieties, but observations on only two varieties are reported in this paper.

OBSERVATIONS ON MARCROSS 6.13

During 1939 three plantings of the variety Marcross 6.13 were observed. The seed, under the name "Whip Marcross," had been obtained from one seedsman. The first of the plantings was a commercial field at Beverly, N. J., and the other two were variety-trial plots, one 2 miles from the commercial planting and the other at New Brunswick, N. J. In each of the first two fields 100 ears were examined on July 6; in the third field 43 ears were examined on July 15.

The results of husk measurements of these ears are summarized in table 1. It was found that the husks in the two fields at Beverly were similar in length but that the husks in the New Brunswick field were much shorter. If the average husk length of ears in the fields near Beverly is considered as having a value of 100, this value for the field at New Brunswick is 44. The factor causing this difference seemed to be one of moisture, since the fields at Beverly were grown during a period of approximately normal precipitation whereas the New Brunswick field was subjected to a severe drought.

TABLE 1.—*Extension of the husks beyond the cob tips of ears of Marcross 6.13 sweet corn in 3 fields at time of roasting-ear harvest in New Jersey, 1939*

Extension of husk beyond the cob (inches)	Ears falling in stated classes of husk extension		
	Commercial field at Beverly	Variety-trial plot at Beverly	Variety-trial plot at New Brunswick
	Percent	*Percent*	*Percent*
3.1 to 3.5		1.0	
2.6 to 3.0	7.0	4.0	
2.1 to 2.5	18.0	19.0	2.3
1.6 to 2.0	29.0	31.0	9.3
1.1 to 1.5	24.0	26.0	20.9
0.6 to 1.0	15.0	15.0	18.6
0.1 to 0.5	5.0	3.0	23.3
0	2.0	1.0	25.6
	Length of husk extension		
	Inches	*Inches*	*Inches*
Longest	2.9	3.4	2.1
Shortest	0	0	0
Average	1.6±0.07	1.6±0.06	0.7±0.09

OBSERVATIONS ON GOLDEN CROSS BANTAM

In 1940 similar observations were made on the variety Golden Cross Bantam near Jobstown, N. J. Six fields were observed all of which had been planted from one lot of seed. Fields 1 to 4, each of about 5 acres, were planted on different dates in equal quarters of one large field, whereas fields 5 and 6, each of about 15 acres, were several miles away although similar in soil type and fertility. The results of husk measurements of ears in these fields are given in table 2.

TABLE 2.—*Extension of the husks beyond the cob tips of ears of Golden Cross Bantam sweet corn in 6 fields at time of roasting-ear harvest, Jobstown, N. J., 1940*

Extension of the husk (inches)	Ears falling in stated classes of husk extension					
	Field 1	Field 2	Field 3	Field 4	Field 5	Field 6
Longer than cob:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
6.1 to 6.5				0.16		
5.6 to 6.0				.16		
5.1 to 5.5				.62	0.24	0.44
4.6 to 5.0		0.40		1.39	.48	1.11
4.1 to 4.5		1.20	1.40	3.42	3.01	5.56
3.6 to 4.0		4.82	4.81	9.16	7.59	17.56
3.1 to 3.5	0.50	10.04	15.23	21.43	14.46	24.89
2.6 to 3.0		15.86	24.85	25.31	30.60	29.55
2.1 to 2.5	3.00	23.09	23.85	22.05	23.50	15.33
1.6 to 2.0	5.50	17.87	18.84	11.80	12.05	4.67
1.1 to 1.5	10.50	14.06	8.22	3.88	6.02	.67
0.6 to 1.0	14.50	8.64	2.20	.62	1.57	.22
0.1 to 0.5	7.50	.40	.20		.12	
Same as cob	37.00	3.62	.40		.36	
Shorter than cob:						
0.1 to 0.5	5.00					
0.6 to 1.0	8.00					
1.1 to 1.5	4.00					
1.6 to 2.0	3.50					
2.1 to 2.5	1.00					
	Length of husk extension					
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
Longest	3.1	4.7	4.5	6.3	5.4	5.4
Shortest	−2.2	.0	.0	.8	.0	.6
Average	0.23±.07	2.11±.04	2.44±.03	2.80±.03	2.64±.03	3.08±.03
Planting date	June 3	June 11	June 18	June 27	July 5	July 13–16.
Date examined	Aug 21	Aug. 23–29.	Sept. 7–9.	Sept. 12	Sept. 24	Oct. 4.
Number of ears	200	498	499	644	830	450.

Extraordinary differences were observed in the length of husks in these fields. If, as seems to be reasonable from many previous observations, the ears of field 5 were of normal development and the average husk length is considered as having a value of 100, the value for the other fields would be as follows: 9 for field 1, 80 for field 2, 92 for field 3, 106 for field 4, and 117 for field 6. Not only were the husks in field 1 much shorter than usual, but more than one-third of them were not longer than the cobs, and more than one-fifth were shorter than the cobs, a condition that is rare in this variety. Ears of this variety showing husks of different lengths with respect to the cob are illustrated in figure 1.

The records indicated that an adverse factor had affected development of husks in field 1 to an extreme degree, and to a much less degree in field 2. Since the summer of 1940 was deficient in precipitation, it seemed likely that lack of moisture might be a responsible factor. Precipitation in the vicinity of Jobstown during June amounted to 1.57 inches, which was 2.21 inches less than normal, and during July to 1.66 inches, which was 3.04 inches less than normal. Rain became more frequent from August 7 to 25, when 3.46 inches fell, but it did not become abundant until August 26 to September 2, when 8.73 inches fell. Therefore, field 1 and to a less degree field 2 were exposed to severe drought during much of the period of ear development.

DISCUSSION

The evidence seemed to point to lack of moisture as the cause of the unusually short husks observed in the plot of Marcross 6.13 at New Brunswick in 1939 and in field 1 of Golden Cross Bantam in 1940. It is probable, however, that the degree of husk shortening may vary with the length and severity of drought encountered by the growing plant, especially during the period when the ears are developing. Since droughts are usually much less severe than the extreme conditions described, the effect on the husks is usually correspondingly less.

It is well known that corn plants subjected to drought become stunted. If the drought is continuous, the husks are usually affected more than the cobs, which grow over a longer period and are therefore more likely to receive the moisture they need to complete their normal development. In the study here reported it was noted that in field 1 of Golden Cross Bantam the husks had completed their growth under dry conditions and were severely stunted, but the subsequent rainfall caused the cobs to make a normal growth so that their length was disproportionate to that of the husks (fig. 1, B). This seems to show that stunting of the plants of this field was limited to those parts that completed growth under the dry conditions and that the parts that grew subsequently to precipitation were more normal.

The rapidity with which earworms penetrate corn ears depends principally on the length and tightness of the husk. Length of the husk beyond the tip of the cob determines the length of the interior silk strands or the abundance of silk that is available as food for the larvae within the ear. Tightness of the husk determines the degree to which the silk strands are pressed together, and this affects the behavior of larvae after they enter the silk mass. If the husk is loose, they may crawl between the silk strands and reach the kernels
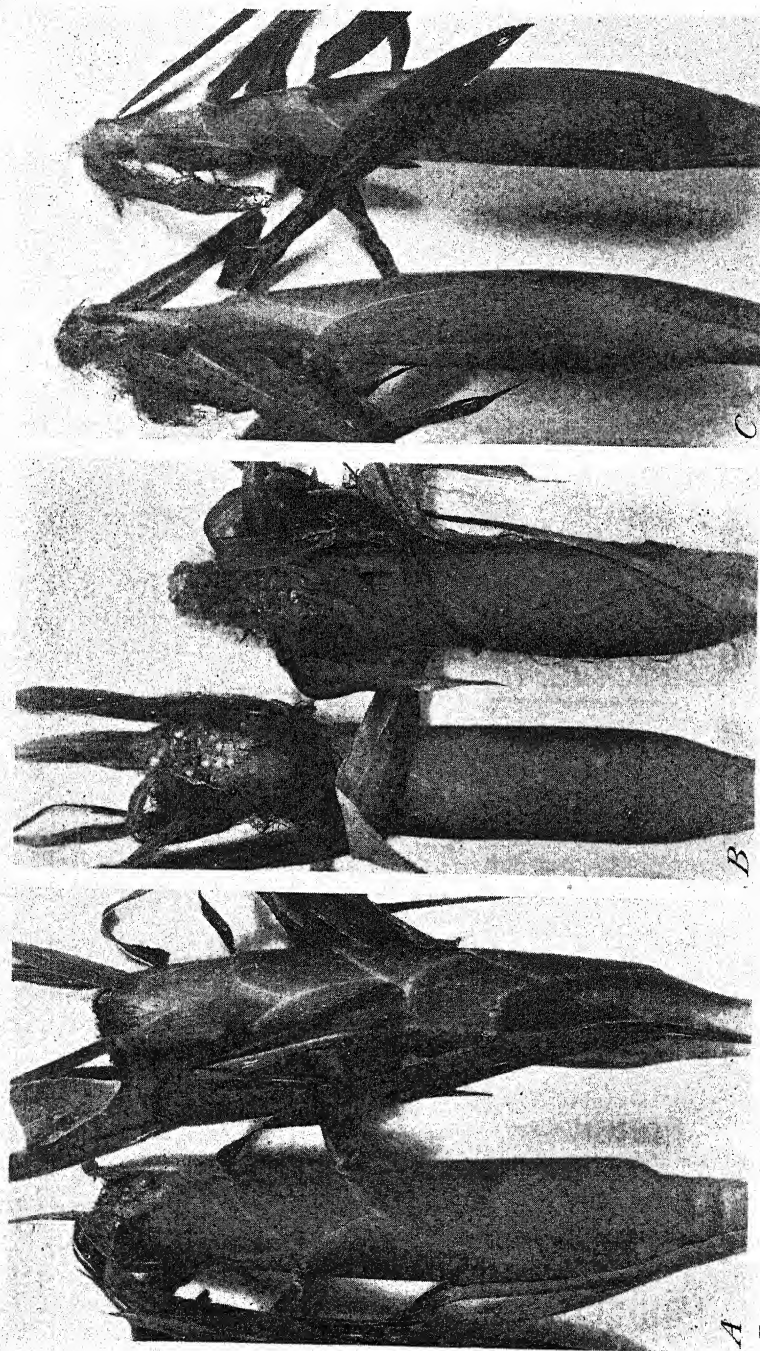
FIGURE 1.—Ears of Golden Cross Bantam sweet corn having (*A*) cobs and husks of equal length, (*B*) cobs longer than the husks, and (*C*) husks 2 to 3 inches longer than the cobs.

soon after hatching, but if the husk is tight the larvae can reach the kernels only by eating a passage through the tightly packed silk. Ears having relatively long, tight husks, in which the larval population is concentrated in the interior silk for several days, can be protected by an insecticidal oil because the entire larval population is reached by it, whereas ears having comparatively short, loose husks, in which many of the larvae are able to disperse to the kernels soon after entering the ears, are not so well protected by the insecticide because it reaches fewer of them. Ears having husks stunted through lack of moisture fall in the latter class, because of the reduced amount and compactness of the silk within the husk tips and the increased limpness and looseness of the husks.

It is thus seen that drought reduces the effectiveness of an insecticidal oil in a variety of sweet corn the husk characters of which under normal conditions are favorable for earworm control. Husk shortening due to drought, although usually much less severe than in the cases cited, has probably been responsible to a considerable degree for the differences that have been observed in the effectiveness of earworm control by means of an insecticidal oil injected into the silk mass.

SUMMARY

The effectiveness of mineral oil containing insecticides applied to sweet corn ears to control earworms (*Heliothis armigera* (Hbn.)) was variable, even when applied to plantings made from the same lot of seed and grown under similar conditions except planting date. Since characters of the husks were known to influence the severity of earworm damage, measurements were made to determine whether differences in extension of the husk beyond the cob occurred which might be responsible for the variations in control. Ears of Marcross 6.13 were measured in 1939 and ears of Golden Cross Bantam in 1940. In each year some of the plantings had been subjected to severe drought, and in fields so affected the husks of ears were much shorter than usual.

In ears having short husks earworms disperse more rapidly than in normal ears and reach the kernels in positions where the insecticidal oil does not reach them, particularly if the husks are limp and do not press tightly against the kernels, as is the case with drought-affected corn.

It appeared that drought injury to corn in causing stunted husks was a factor determining the variable control that had been observed.

POLLINATION AND SEED FORMATION IN GRASSES [1]

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INTRODUCTION

An understanding of the mode of pollination and seed formation of a species is commonly accepted as fundamental in the initiation of any plant breeding program. The objective of the work reported herein was to obtain such information for a number of exotic and endemic grasses of agronomic interest and importance in the Pacific Northwest. Many of the species reported upon here have been studied only superficially, but until more complete information is available the data may be of use to those concerned with improvement and propagation problems. While strain comparisons were not made, the results for each species represent more than one strain or collection in most instances.

In recent years considerable attention has been given to studies of fertilization and subsequent embryological development of certain grasses. The occurrence of apomixis in some species raises the question whether seed setting under bags should be considered as necessarily identical with self-fertility in the true genetic sense. To answer this question definitely would require cytological and embryological investigations, and in the work here discussed such studies were not made. However, high incidence of seed setting under bags may be considered practically as an approximate criterion of breeding behavior. In this paper the term "fertility" is used only in the sense of seeds developed, with no implication as to whether true fertility existed.

REVIEW OF LITERATURE

Except for certain general relations and methodology, only work pertinent to the fertility of the species under investigation will be reviewed here.

A summary of some of the results of earlier investigations of self-fertility in a number of grass species is presented in table 1. In some instances the reports are based upon observations of flowering rather than upon controlled pollination studies. The data for some species allow considerable variation in interpretation, and the classes as given should be considered as general only. However, as the table indicates, there is essential agreement among the different workers as to relative self-fertility. If heritable plant or strain variations were noted, this fact is indicated. Peto (*24*) [2] reported Malte, Robinson, and Kirk as believing species of *Agropyron* to be highly self-fertilized, and Beddows (*2*) found annual species to be generally highly self-fertile.

TABLE 1.—*Results of other investigators in self- and cross-fertility studies of certain grass species*

Species	Self-fertility			Heritable plant or strain variation
	High	Intermediate	Low	
Agropyron caninum	¹(2)			
Agropyron cristatum			(31)	(31).
Agropyron elongatum			(1, 11, 30)	(11).
Agropyron intermedium			(30)	
Agropyron repens			(2)	(2).
Agropyron tenerum	(2, 7, 13, 19)			(2).
Arrhenatherum elatius			(4, 5, 29)	(29).
Bromus carinatus	(2)			
Bromus erectus			(2, 16)	
Bromus inermis			(2, 7, 12, 14, 29, 31)	(2, 7, 12, 14, 31).
Bromus madritensis	(2)			
Bromus mollis	(16, 29)			
Bromus secalinus	(2, 16)			
Bromus tectorum	(2, 16)			
Dactylis glomerata	(23)		(4, 5, 7, 16, 18, 20, 27, 29, 32)	(4, 7, 18, 23, 29).
Elymus junceus		(26)		
Festuca arundinacea		(2, 31)	(29)	(2, 31).
Festuca elatior		(2, 16, 31)	(4, 5, 7, 23, 29)	(2, 7, 23, 29, 31) ⁷
Festuca ovina			(29)	
Festuca rubra			(8, 16, 22, 23, 29, 31)	(8, 22, 23, 31).
Hordeum bulbosum			(16, 17)	
Phalaris arundinacea			(29)	
Poa compressa	(31)	(29)		
Poa pratensis	(16, 22, 23)	(29)	(5)	(22, 23, 29).

¹ Numbers refer to Literature Cited, p. 16.

Raum (*25*) and Fruwirth (*5*) early recognized the relation of environmental conditions to flowering, pollination, and seed set. Type of inflorescence, covering material, and size of enclosure were given particular attention. Knoll (*15*) found a good negative correlation between humidity and seed set. Beddows (*2*) concluded that some depression of seed set occurred because of bagging. Hayes and Barker (*6*) observed fertility under greenhouse conditions and under field conditions to be positively correlated.

Raum (*25*), in studying some of the problems of technique such as pollination control, compared "pergamine," oil-paper, cotton, and gauze coverings. Fruwirth (*5*) compared pergamine bags and isolation of two or more plants of the same clone together under oilcloth-covered frames, with respect to seeds produced. While the data presented were not for comparable years and the results were variable, oil paper or cloth was preferred to pergamine because of durability. Frandsen (*4*), in tests with pergamine, linen, and glass coverings, found that fewest seeds developed under pergamine. McRostie, as reported by Wolfe and Kipps (*32*), found that cloth cages allowed higher seed set than glassine bags. Knoll (*15*) obtained higher seed set with pergamine than with oil or paraffin paper; the pergamine allowed more light to enter the bag. Jenkin (*10*) secured better results with parchment than with glazed or waxed paper. Beddows (*2*) utilized parchment sleeves or closely woven cloth bags for pollinations out of doors and glazed paper bags in the greenhouse. Nilsson (*23*) used double pergamine bags.

In general Frandsen (*4*) obtained somewhat better seed setting when more than one inflorescence was enclosed in the same bag, although in some instances the differences were slight. Similar results were obtained by Sylven as reported by Nilsson (*23*). Knoll (*15*) found isolation of panicles to be more favorable for seed set than enclosure

of entire plants. Wolfe and Kipps (*32*) obtained higher seed set by covering heads with paper bags than by enclosing entire plants with cloth cages. Clarke (*3*) recognized the possibility that differential reaction to coverings might exist among plants.

Fruwirth (*5*) observed considerable variation in seed setting among different heads of the same plant, and similar observations were made by Clarke (*3*), Hayes and Garber (*7*), and Nilsson (*23*).

Marked interannual variations in the ability of plants to set seed have been reported by Fruwirth (*5*), Frandsen (*4*), Jenkin (*9, 10*), Nilsson (*23*), and Schultz (*27*). Fruwirth (*5*) and Jenkin (*9*) have also called attention to the inadequacy of single or few tests of self-fertility as a basis for classifying individuals in this respect. Fruwirth has noted large differences among plants of the same clone. Nilsson (*23)* found variation between clonal families to be greater than that within clonal families. He also observed a fair to good relationship between results of pollination of the same plants in different years.

Frandsen (*4*) was one of the earliest workers to report differences in self-fertility among strains of the same species. Similar results were reported by Hayes and Barker (*6*) and Beddows (*2*). Variation within strains in ability to set seed under conditions of selfing has also been noted by several workers. Sylven, cited by Nilsson (*23*), used the highest values obtained as the result most descriptive of true fertility. Nilsson, however, considered the average to be more representative of the individual or strain concerned.

Few reports are available in which the germination of seeds from cross-pollination is compared with that of seeds derived from selfing. Jenkin (*9*) found that seeds from selfed plants germinated poorly as compared with those obtained from cross-pollination, but Wolfe and Kipps (*32*) observed no difference.[3]

MATERIAL AND METHODS

Specific identification of all the species studied was verified in the course of the investigations, during the seasons from 1936 to 1940, inclusive.[4] The close relationship of *Bromus carinatus* Hook. and Arn. to certain other species listed is recognized. An analogous situation may occur in *Poa ampla* Merr. and species similar to it. Numbered strains were used, some being foreign plant introductions and a few derived from commercial sources. Many were collections of native species made in the Pacific Northwest by the Division of Nurseries of the Soil Conservation Service, United States Department of Agriculture, and subsequently propagated at Pullman, Wash. All strains were not studied in all years.

General conditions for flowering and seed setting during the seasons of the investigations appeared to be favorable, though no detailed observations were made of them and brief exceptions may have occurred. Day temperatures rarely reached 100° F., with 65° to 85° common; temperatures at night were low, ranging from 40° to 70°. Humidity was typical of the Columbia Basin area, being generally low during the day and relatively high in very early morning. Moisture conditions were favorable for growth and seed production;

occasional rains were interspersed with relatively dry, often clear weather. The average mean-maximum and mean-minimum temperatures and the average precipitation for May, June, and July (1936–40), when flowering and ripening occur, are shown in table 2.

TABLE 2.—*Average mean-maximum and mean-minimum temperatures and average precipitation for May, June, and July, 1936–40*

Item	May	June	July
Temperature:			
Minimum_____°F___	43.32	43.97	49.74
Maximum_____°F___	69.97	70.53	84.94
Precipitation_____inches__	1.48	1.28	0.46

The soil upon which the plants were growing was a medium-heavy Palouse silt loam of somewhat depleted fertility but capable of producing 20 to 35 bushels of wheat per acre. Except annuals, all plants utilized for the studies were in the second to fourth growth years. During the period of investigation no insect or disease injuries appeared to be seriously affecting the growth of bagged plants.

The materials used in covering inflorescences were parchment paper, kraft paper, and glassine bags, or tissue-paper wrappings. The parchment paper was used most frequently. The tissue wrappings were used for certain species, as *Agropyron cristatum* and others having dense, spikelike heads; or those, like *Arrhenatherum elatius*, with slender peduncles that are easily broken. Beginning at the wide end of paper cut in a long triangle, and folding the upper corner down, the inflorescence was wrapped snugly toward the base and the lower end was secured by light string. Heads thus wrapped were unsupported. Before anthesis inflorescences were enclosed with only one per bag, a wisp of cotton being inserted at the mouth of the bag and around the culm. This was held in position by the string tying the bag, and all bagged inflorescences were stabilized by tying them to bamboo-cane supports. Coverings were not removed until determination of seed set following harvest. Open-pollinated inflorescences, usually three in number, were used as checks. As a rule five heads per plant were bagged. In some species shattering occurred in many unbagged heads.

The number of florets per head was estimated by approximate counting. The head was then threshed by hand and the resulting mass was examined for seeds, this process being facilitated by the use of reflected light. Heads with broken or otherwise damaged culms were not included.

RESULTS

GENERAL FERTILITY

In table 3 are presented summary data for seed setting of a number of species with which artificial isolations were made under field conditions. The generally low average of seed set in all species is due in part to a high floret count, as all reasonably well developed florets were included and many of those in secondary and tertiary positions often do not produce seeds under ordinary conditions. In some instances the average seed set per floret was greater with self- than with open-pollination. This is believed to be due to shattering of the seeds of the latter class, though it might be due in part

to random fluctuation.   One hundred percent is assumed to be the maximum seed set for selfed inflorescences.   In several species the selfed seed percentage approached 100, and such species are considered to be highly or completely self-fertile.   Such a conclusion may be verified in part if single-plant progenies appear to breed true to the parental type.   Table 3, column 5, shows the relative fertility class of the different species on the basis of breeding behavior in tests of progeny uniformity.   In a few species the amount of selfed seed is less than would be expected on the basis of self-fertility, but breeding behavior supported the latter classification.   To illustrate, progenies of *Poa ampla, P. juncifolia,* and *P. nevadensis* appeared to be highly uniform, though large variations occurred in the amount of seed produced.

TABLE 3.—*Relative seed setting of a number of grass species self- and cross-pollinated under field conditions*

[Data from several seasons]

Species	Plants	Average seeds per floret		Self-seed
		Self-pollinated	Cross-pollinated	
	Number	*Number*	*Number*	*Percent*
Agropyron caninum (L.) Beauv	14	0.588	0.735	[1] 80.00
Agropyron ciliare (Trin.) Franch	5	.892	.950	[1] 93.89
Agropyron cristatum (L.) Gaertn	250	.006	.277	[3] 1.99
Agropyron dasystachyum (Hook.) Scribn	4	.011	.172	[3] 6.40
Agropyron desertorum (Fisch.) Schult	5	.006	.579	[3] .97
Agropyron elongatum (Host) Beauv	11	.090	.333	[2] 27.16
Agropyron inerme (Scribn. and Smith) Rydb	45	.018	.286	[3] 6.22
Agropyron intermedium (Host) Beauv	9	.007	.234	[3] 2.99
Agropyron repens (L.) Beauv	10	.005	.248	[3] 2.10
Agropyron sibiricum (Willd.) Beauv	18	.001	.551	[3] .15
Agropyron smithii Rydb	42	.010	.457	[3] 2.08
Agropyron spicatum (Pursh) Scribn. and Smith	51	.015	.294	[3] 5.03
Agropyron trachycaulum (Link) Malte	36	.669	.589	[1] 100.00
Agropyron trichophorum (Link) Richt	35	.012	.227	[3] 5.33
Arrhenatherum elatius (L.) Presl	47	.019	.399	[3] 4.74
Bromus carinatus Hook. and Arn	10	.552	.302	[1] 100.00
Bromus erectus Huds	20	.033	.355	[3] 9.36
Bromus inermis Leyss	199	.028	.375	[3] 7.39
Bromus marginatus Nees	10	.890	.975	[1] 91.28
Bromus polyanthus Scribn	21	.621	.674	[1] 92.13
Dactylis glomerata L	50	.038	.417	[3] 9.21
Elymus angustus Trin	2	.090	.680	[3] 13.24
Elymus canadensis L	17	.599	.943	[1] 63.54
Elymus chinensis (Trin.) Keng	2	.003	.100	[3] 3.30
Elymus condensatus Presl	6	.005	.383	[2] 1.30
Elymus giganteus Vahl	6	.005	.231	[3] 1.92
Elymus glaucus Buckl	19	.661	.629	[1] 100.00
Elymus junceus Fisch	12	.002	.593	[3] .35
Elymus sibiricus L	2	.367	.667	[1] 55.00
Elymus triticoides Buckl	10	.001	.128	[3] .39
Festuca elatior L	9	.034	.171	[2] 20.12
Festuca elatior var. *arundinacea* (Schreb.) Wimm	10	.170	.651	[2] 28.07
Festuca idahoensis Elmer	20	.153	.347	[2] 44.17
Festuca ovina L	17	.025	.284	[3] 8.86
Festuca rubra L	3	.047	.177	[2] 26.57
Hordeum brevisubulatum (Trin.) Link	6	.001	.634	[3] .11
Hordeum bulbosum L	22	.045	.758	[3] 5.92
Koeleria cristata (L.) Pers	15	.001	.279	[3] .21
Oryzopsis hymenoides (Roem. and Schult.) Ricker	14	.284	.427	[3] 5.63
Phalaris arundinacea L	20	.018	.429	[3] 4.29
Phleum graecum Boiss and Heldr	5	.006	.765	[3] .72
Phleum phleoides (L.) Karst	5	.002	.850	[3] .20
Poa ampla Merr	20	.566	.668	[1] 84.76
Poa canbyi (Scribn.) Piper	29	.650	.624	[1] 100.00
Poa compressa L	4	.441	.735	[1] 59.98
Poa juncifolia Scribn	3	.186	.590	[1] 31.47
Poa nevadensis Vasey	6	.283	.509	[1] 55.63
Poa pratensis L	6	.533	.814	[1] 65.48

See footnotes at end of table.

TABLE 3.—*Relative seed setting of a number of grass species self-and cross-pollinated under field conditions*—Continued

| Species | Plants | Average seeds per floret | | Self-seed |
		Self-pollinated	Cross-pollinated	
	Number	*Number*	*Number*	*Percent*
Puccinellia distans (L.) Parl	4	0.029	0.660	[3] 4.38
Puccinellia nuttalliana (Schult.) Hitchc	9	.190	.491	[2] 38.67
Sitanion hystrix (Nutt.) J. G. Smith	6	.396	.823	[1] 48.13
Stipa columbiana Macoun	22	.838	.730	[1] 100.00
Stipa comata Trin. and Rupr	5	.353	.521	[1] 67.78
Stipa lettermani Vasey	2	.606	.554	[1] 100.00
Stipa robusta Scribn	2	.596	.540	[1] 100.00
Stipa viridula Trin	2	.886	.868	[1] 100.00

[1] High self-fertility.
[2] Intermediate self-fertility.
[3] Low self-fertility.

Seed setting under greenhouse conditions was determined for several species as follows:

Species:	Seeds per floret
Agropyron caninum (L.) Beauv	[1] 0.878
Agropyron inerme (Scribn. and Smith) Rydb	[3] .014
Agropyron intermedium (Host) Beauv	[3] .133
Agropyron semicostatum (Steud.) Nees	[1] .641
Bromus catharticus Vahl	[1] .971
Bromus commutatus Schrad	[1] .945
Bromus inermis Leyss	[3] .007
Bromus madritensis L	[1] .801
Bromus marginatus Nees	[1] .983
Bromus mollis L	[1] .940
Bromus secalinus L	[1] .610
Elymus canadensis L	[1] .868
Elymus sibiricus L	[1] .749
Elymus virginicus L	[1] .978
Festuca elatior var. *arundinacea* (Schreb.) Wimm	[3] .000
Hordeum brevisubulatum (Trin.) Link	[3] .008
Hordeum jubatum L	[1] .720
Hordeum murinum L	[1] .884
Hordeum nodosum L	[2] .388
Secale montanum Guss	[3] .630
Sitanion hystrix (Nutt.) J. G. Smith	[2] .486

[1] High self-fertility.
[2] Intermediate self-fertility
[3] Low self-fertility.

Single plants were grown in spatial isolation without covering. The results, where comparable, are in general agreement with those for plants grown under field conditions except possibly for *Festuca elatior* var. *arundinacea*. Observations of other plants fruiting in the greenhouse—of which no accurate counts were made—indicated that *Bromus carinatus, B. marginatus, B. rigidus* Roth., *B. tectorum* L., and *Hordeum nodosum* were also highly self-fertile, although this finding is not in complete agreement with the data shown in table 3 for the last-named species. *Agropyron elongatum* and *Secale montanum* set few seeds, and *Hordeum brevisubulatum* was sterile.

In table 4 distributions into fertility classes have been made for plants studied under field conditions. In many instances but few individuals were observed, and distributions for such species may have comparatively little significance. The table includes only those species of which nine or more plants were studied.

TABLE 4.—*Comparison of self- and open-pollinated progenies in distribution of plants in fertility classes based upon several years' results*

Species	Pollination	Plants setting indicated number of seeds per floret — 0.00	0.025	0.075	0.125	0.175	0.225	0.275	0.325	0.375	0.425	0.475	0.525	0.575	0.625	0.675	0.725	0.775	0.825	0.875	0.925	0.975	1.000	Total plants
Agropyron caninum	S	1				2		1										1	3	2	2			14
	O				2	2											1	1	2	1	2	2	1	14
Agropyron cristatum	S	92	141	6	4	29		14	16	2	17	11	1	1	1									250
	O	33	22	18	22	18	18										1							
Agropyron elongatum	S	3			1																			11
	O																							
Agropyron inerme	S	19	20	4	6	1		4	4	1	2	2			4	1		2	2					45
	O		5	6	6	8	2	1					2		1	1				2	2			45
Agropyron intermedium	S	1	8					1		1														9
	O		1						2					2										9
Agropyron repens	S	6		1	2					1														10
	O		3	2																				10
Agropyron sibiricum	S	14	4	3	2	3		2	1	1	1	4	2	1	2		1							18
	O																		2				1	18
Agropyron smithii	S	14	24											4	2									42
	O	1	1				5								1	2				2		1		42
Agropyron spicatum	S	25	20	4	2	1	4	2	4	2	7	5	2	2	1									51
	O		7		8	3	4	3	5			2				1								51
Agropyron trachycaulum	S	1			4	1	5	7	6	7	4	1	3	1				1						36
	O				4								1	1										36
Agropyron trichophorum	S	18	15	5	1			2	1	2	1	1	2	3	1				2					35
	O		3	6	3	8	5										1							35
Arrhenatherum elatius	S	6	34		1			3	5		2	1	5	2		3	1	1	2					47
	O		1		4								2	2										47
Bromus carinatus	S																							10
	O		13	3	2	10		5	1	3	3	2				2	2	1						10
Bromus erectus	S	40				1		1	4	2	4		1		1		1		1					19
	O			3	2	2	1	3	1															19
Bromus inermis	S	126		17	7	4	1	17	14	26	24	13	27	6	10	7		3						199
	O	3		8	14	13	11											1	1					199
Bromus marginatus	S					1								3	3	2	1	3	1	1		8		10
	O													2				4				4		10
Bromus polyanthus	S									1	1	2	2					2	2	3	2			21
	O										1	1	3					1	1					21
Dactylis glomerata	S		30					1	9		4	4	8	3	2	4	1		1					50
	O		1						2			2	2	2		2	2							50
Elymus canadensis	S			7						1								1			2	1		17
	O		2					3		5						1						2		17
Elymus glaucus	S	2			2	1	1		1		1			1	1				1	1	2		10	19
	O						2			3					1	5							3	19
Elymus junceus	S	12																						12
	O							1				1			3	2		1			2		1	12

TABLE 4. — *Comparison of self- and open-pollinated progenies in distribution of plants in fertility classes based upon several years' results* —Con.

Species	Pollination	Plants setting indicated number of seeds per floret																						Total plants
		0.00	0.025	0.075	0.125	0.175	0.225	0.275	0.325	0.375	0.425	0.475	0.525	0.575	0.625	0.675	0.725	0.775	0.825	0.875	0.925	0.975	1.000	No.
		No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
Elymus triticoides	S	8	2																					10
	O		1	1	5	2			1															10
Festuca elatior	S		1	1																				9
	O		4	5			7																	9
Festuca elatior var. arundinacea	S	2	1																					10
	O																	1	1	1	2	1	1	10
Festuca idahoensis	S		3																					20
	O		2								2		1	1	1	1	1	1						20
Festuca ovina	S	5	10						1															17
	O		1						1		1													17
Hordeum bulbosum	S	7	6																	4				22
	O													2			4	7						22
Koeleria cristata	S	9	6						5				1											15
	O							3	3	1	1	1					1	1		1				15
Oryzopsis hymenoides	S	1	19																					14
	O							1	1		2	1		1			1	1	3					14
Phalaris arundinacea	S		1	2	2	2	1	1	3	1	3	1		1	2	1	2	1						20
	O			2	1	5	2	1	1	1	1	1	1	1	1	1	1	2	3	6	6		3	20
Poa ampla	S				1	2																	2	20
	O				2								1	4	1	2	3	3	2	2	1		1	20
Poa canbyi	S		1	2	1		1		1		1		2	1	2	5	1	1	3	6		2	1	29
	O	5	2		1								1	4		2	6	3	3	2	1	3	1	29
Puccinellia nuttalliana	S	5	2	1	1											1		2						9
	O					2									2	6								9
Stipa columbiana	S						1												3	3	2	2		22
	O										1									5	3	3		22

Among species previously classified as highly self-fertile, *Agropyron caninum, A. trachycaulum, Elymus canadensis, E. glaucus, E. sibiricus, Poa ampla, P. canbyi, P. compressa, P. juncifolia, P. nevadensis, P. pratensis,* and *Stipa comata* included some plants lower in self-fertility than might have been expected. It should be noted, however, that few plants of some of these species were studied. In species with low average self-fertility the distribution of individual plant values is well concentrated in lower classes. In *Bromus inermis, Dactylis glomerata,* and *Oryzopsis hymenoides* more variation occurred among individual plants. Although plants of most species having a low average self-fertility were well grouped, occasional medium-fertile individuals occurred. This may be seen for *Agropyron cristatum, A. inerme, A. smithii, Dactylis glomerata,* and *Oryzopsis hymenoides.* In these experiments, species of most uniformly low self-fertility were *A. sibiricum, Elymus junceus, E. triticoides, Hordeum brevisubulatum, Koeleria cristata,* and *Phleum phleoides.*

SEASONAL AND OTHER RELATIONS

Table 5 includes data for seed setting with and without parchment-paper bags for the same plants of four species observed in several years. Great differences are apparent when results for different species, self- and open-pollination, and different years are compared. A summary was made of the regularity of zero seed set, based upon other data of *Agropyron cristatum, Bromus inermis,* and *Dactylis glomerata* when selfed for several years. Plants giving zero seed set in 1 year showed a strong tendency to continue to fail to produce seed, though many exceptions occurred. The comparisons of plant behavior from year to year among individuals of low self-fertility indicate the relative stability of this character to be good from season to season.

Attempts were made to correlate the results for successive years in seed setting with self- and open-pollination in several species. These results were derived from data from 35 plants of each of 6 species. Plants failing to produce seed when selfed or open-pollinated were not included. The relations of selfed seed percentages as obtained for the same plants in 2 different years are given in table 6.

TABLE 5.—*Interannual variation of seed setting of individual selected plants with self- and open-pollination*

AGROPYRON CRISTATUM

Plant No.	Average seeds per floret							
	Open-pollinated				Selfed			
	1937	1938	1939	1940	1937	1938	1939	1940
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
H4-1	0.06	0.14	0.34	0.10	0.005	0.000	0.004	0.003
H13-3	.22	.24	.37		.000	.000	.002	
H14-2	.55	.49	.52		.003	.020	.004	
H16-2	.25	.19	.16		.003	.000	.002	
H17-2	.005	.01	.00		.000	.000	.000	
H17-3	.16	.12	.10		.000	.000	.007	
H19-1	.12	.25	.17		.000	.000	.001	
H21-2	.17	.43	.21	.58	.005	.001	.000	.120
H22-2	.19	.30	.24		.000	.002	.002	
H26-2	.01	.04	.01		.000	.000	.000	

TABLE 5.—*Interannual variation of seed setting of individual selected plants with self- and open-pollination*—Continued

ARRHENATHERUM ELATIUS

Plant No.	Average seeds per floret							
	Open-pollinated				Selfed			
	1937	1938	1939	1940	1937	1938	1939	1940
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
12	0.38	0.14	0.19	0.47	0.020	0.007	0.010	
18	.26	.12	.67		.040	.060	.026	
26	.50	.18	.73		.007	.060	.008	
31	.40	.16	.80		.080	.010	.004	
38	.34	.16	.39		.040	.002	.010	
50	.58	.18	.50		.007	.020	.009	
55	.54	.18	.67		.020	.060	.001	
61	.54	.14	.74	.58	.000	.000	.001	0.005
80	.82	.20	.75		.014	.000	.007	
86	.16	.16	.67		.040	.014	.005	

BROMUS INERMIS

	1936	1937	1938	1939	1936	1937	1938	1939
88	0.12	0.32	0.34	0.48	0.000	0.000	0.001	0.000
90	.16	.26	.19	.27	.001	.000	.000	.000
91	.14	.40	.49	.42	.016	.100	.006	.017
92	.16	.58	.53	.38	.000	.060	.004	.000
93	.06	.58	.68	.33	.006	.008	.006	.000
94	.14	.38	.44	.39	.120	.032	.008	.005
95	.10	.50	.18	.28	.002	.002	.000	.000
96	.06	.40	.70	.50	.000	.016	.026	.006
100	.10	.24	.16	.23	.006	.004	.002	.000
102	.08	.44	.38	.43	.002	.000	.030	.049

DACTYLIS GLOMERATA

11		0.20	0.56	0.42		0.000	0.000	0.030
29		.40	.57	.67		.020	.035	.070
31		.17	.52	.69		.003	.000	.030
38		.56	.68	.49		.002	.000	.030
51		.43	.50	.49		.005	.007	.020
63		.30	.35	.53		.020	.005	.080
65		.30	.36	.39		.410	.042	.050
72		.37	.54	.31		.001	.000	.004
77		.31	.54	.51		.070	.000	.010
93		.33	.41	.46		.050	.000	.090

TABLE 6.—*Percentages of seed obtained from the same self-pollinated plants in different years*

Species	r	P
Agropyron cristatum	0.50	>0.01
Agropyron inerme	.00	
Arrhenatherum elatius	.42	>0.05
Bromus inermis	−.04	(1)
Dactylis glomerata	.07	(1)
Poa ampla	.44	(1)

[1] Insignificant.

Of the species listed, *Poa ampla* is considered to be highly self-fertile. When seed setting with open-pollination was compared in consecutive years, significant agreements were indicated for *Arrhenatherum elatius*

and *P. ampla.* Insignificant *r* values were obtained for *Bromus inermis* and *Dactylis glomerata.* When results in seed setting from self- and open-pollination were compared for the same season, negative correlations were obtained for *Agropyron cristatum, Arrhenatherum elatius, Bromus inermis,* and *Dactylis glomerata.* That for *A. elatius* was highly significant, *P* values for the others not reaching the 0.05 point.

While data were available for other species, these were generally insufficient to permit comparable analyses. The point of principal interest in the results presented is a frequent failure in close agreement of results in different seasons.

In *Agropyron cristatum* and *Bromus inermis*, where data were available for more plants, the chi-square test for independence was utilized to study the relation of self- and cross-fertility. In this test plants failing to set seed when selfed but which were cross-fertile were included. The results are given in table 7.

TABLE 7.—*Statistical analysis of data on relation of self- and cross-fertility*

Species	Plants	Degrees of freedom	x^2	P
	Number			
*Agropyron cristatum*_____	294	12	56.04	>0.01
*Bromus inermis*_____	207	9	34.61	>.01

The analysis indicates that self- and cross-fertility are unquestionably interrelated in a positive manner in these species.

Coefficients of variability indicated that plant variation in seed setting was much greater with self- than with open-pollination, as might be expected. The variation was not closely similar for all species or in all seasons. Neither did variability in seed setting always move in the same direction from season to season when results from self- and open-pollination for the same plants were compared.

It should be noted that in the previous discussion, with some exceptions, plants failing to set seed in any year have been omitted from consideration. While this procedure may be somewhat arbitrary, since by random chance some partly fertile plants may fail to set seeds, a classification is required. Percentages of self-sterile plants for several species were as follows: *Agropyron cristatum,* 36.8; *Arrhenatherum elatius,* 13.0; *Bromus inermis,* 16.0; and *Dactylis glomerata,* 20.0.

Eighteen species, principally of *Agropyron* but including others, of which more florets were counted, were studied for relative seed set under different types of covering. Considering all 18 species, seed set was highest under parchment-paper bags in 1 of 2 comparisons. In comparisons of tissue wrappers and glassine bags, the latter gave the higher results in 9 comparisons, and the former in 4; in 4 the results were similar.

Comparisons between parchment- and kraft-paper bags for covering were made with *Bromus inermis,* sister clones being used as replicates. The results are given in table 8.

Table 8.—*Relative seed set with parchment- and kraft-paper bags for coverings*

Covering	Florets	Seeds	Average seeds per floret
	Number	*Number*	*Number*
Parchment	11,659	107	0.009
Kraft	12,181	32	.003

The seed set under parchment-paper bags exceeded that under kraft-paper bags in 14 of 16 plants studied. In another season, seed set in glassine bags exceeded that in parchment-paper bags in 12 of 21 trials, results with parchment paper exceeding those with glassine but 4 times. In some instances intraclonal variation was as high as 900 percent. In earlier investigations parchment paper was generally superior to kraft paper or glassine as a covering in tests with many species.

A limited number of studies were made to determine the seed development in *Bromus inermis* where 1 or more than 1 inflorescence was enclosed in each parchment-paper bag. The results are summarized in table 9. Evidently humidity or such other effects as may result from placing 2 or more heads in 1 bag had no important influence on seed setting in *B. inermis* under the conditions of this experiment, though averages suggest differences in favor of more panicles up to 5 being enclosed in each bag. In counting the possible comparisons, the average of 2 or more panicles exceeded that of 1 in seed set 36 times, was exceeded by one 32 times, and was the same 8 times.

Table 9.—*Comparison of seed setting in Bromus inermis with 1 or more than 1 panicle enclosed in each bag, 1940*

Panicles enclosed (number)	Total florets	Total seeds	Average seeds per floret
	Number	*Number*	*Number*
2	565	70	0.124
1 [1]	285	15	.053
3	3,991	285	.071
1	1,480	50	.034
4	21,081	779	.037
1	5,919	127	.021
5	16,619	424	.026
1	4,068	258	.063
Total or average:			
More than 1 panicle enclosed	42,256	1,558	.037
1 panicle enclosed	11,752	450	.038

[1] Check for number above.

Germination percentages of seeds resulting from self-pollination and open-pollination in 3 years have been summarized for some species in table 10. Germination from self-pollination exceeded that from open-pollination twice in 1936, 7 times in 1938, and once in 1939, for a total of 10 in 30 comparisons. Differences within species were inconsistent. In general the data indicate that selfed seeds may not germinate so well as those from open pollination. It should be noted that the species listed are from the group of low self-fertility, as self-fertile species might be expected to germinate equally well from selfed or open-pollinated seed.

TABLE 10.—*Summary of germination percentages of seeds of species self-pollinated and open-pollinated in several years*

Species	Seeds germinated from self-pollination or open pollination							
	1936		1938		1939		Average	
	Self	Open	Self	Open	Self	Open	Self	Open
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Agropyron cristatum	33.4	41.1	56.6	37.8	47.1	55.6	45.7	44.8
Agropyron inerme	54.6	71.1	53.1	50.3	67.8	68.0	58.5	63.1
Agropyron smithii	91.7	63.0	85.7	32.3	43.2	53.0	73.5	49.4
Agropyron spicatum	61.0	67.7	38.8	43.2	72.3	80.7	57.4	63.9
Agropyron trichophorum	47.1	59.4	53.8	23.9	9.3	22.2	36.7	35.2
Arrhenatherum elatius	60.7	48.1	44.4	89.6	11.5	20.7	38.9	52.8
Bromus erectus			26.0	26.8	77.5	75.5	51.8	51.2
Bromus inermis	58.9	65.5	29.6	22.2	50.7	77.6	46.4	55.1
Dactylis glomerata			24.2	21.9	10.1	34.8	17.2	28.4
Hordeum bulbosum			59.4	41.8	24.7	51.4	42.1	46.6
Phalaris arundinacea	43.6	82.6	5.8	14.6	36.9	47.0	28.8	48.1
Average	56.4	62.3	43.4	36.8	41.0	53.3	45.2	49.0

DISCUSSION

Nilsson (*23*) presented a thorough discussion of the numerous factors and relationships involved in self-fertility studies of grasses. The writer is in general agreement with his conclusions and viewpoint.

The conditions under which the present work was done were considered to be generally favorable for seed setting. This conclusion is based to a large extent upon the fact that under conditions at Pullman, Wash., practically all grass species show a high degree of fertility under field conditions and seed yields are very good. It is admitted, however, that short periods unfavorable to seed setting might markedly affect the results with specific strains. The possible effects of bringing together a number of ecological strains of a species or species complex and studying these under one environment should also be recognized, for such strains might be modified differentially in fertility as well as in other characters.

The low average fertility values presented may be attributed to high estimates of floret numbers, since all reasonably well developed flowers were counted. In self-fertile species practically all of the primary florets developed seeds. Beddows (*2*) reported, however, that empty florets were not restricted to any part of the inflorescence.

The data presented will allow the reader to arrive at his own conclusions as to the self-fertility of the species studied. Rogler (*26*) reported *Elymus junceus* to be of intermediate self-fertility, whereas very few selfed seeds were obtained for this species in these experiments. *Agropyron elongatum* and *Festuca rubra* were reported by others to have low self-fertility, whereas they were found to be somewhat intermediate in the present investigations. The results with *Poa pratensis* were in agreement with those of Nilsson (*23*). It is possible that apomixis may be concerned in seed formation in species other than *Poa pratensis*, where it is known to occur commonly. With results for all other species previously studied the work reported here was in agreement.

The self-fertility studies have served to verify, in the main, the conclusions reached by observing breeding behavior, namely, that

a number of native species in the Pacific Northwest are highly self-fertile. Since these occur widely throughout the area, collection of types from local habitats [5] has brought together numerous varieties that may be expected to breed true or practically so. Such have been the results in trials thus far. Classification of other species of intermediate or low self-fertility will serve to aid in the development of suitable breeding and propagation systems where these species are of interest. However, it has been observed that native collections of some species such as *Agropyron inerme* and *A. spicatum* show unexpected uniformity in plant rows if high self-sterility is assumed. This is particularly noticeable in early years of propagation from native sources and may be due in part to the relative uniformity of ecotypes, though cross-pollination may occur within the type. Intertype sterility may also account, in part, for the tendency toward preservation of relative uniformity in some strains.

Considering all plants of *Agropyron cristatum* and *Bromus inermis* studied, self- and cross-fertility appeared to be positively related, while the negative relation in *Arrhenatherum elatius* was hardly expected. Nilsson (*23*) found variations in self-fertility to be positively related to general fertility and discussed the reasons why this might occur. Some types of self-fertility were shown to be independent of general fertility.

Parchment-paper bags were considered to be most satisfactory for controlling pollination, though advantages in seed setting were not great or entirely consistent. In many highly self-fertile species bagged heads set fewer seeds than those unbagged, suggesting the presence of some detrimental influence due to bagging.

Nilsson (*23*) pointed out that true self-fertility is probably somewhat higher than that indicated in controlled-pollination tests. This would appear to be a justifiable conclusion, since enclosure of flowers might ordinarily be detrimental to seed setting, as suggested by Raum (*25*), Fruwirth (*5*), Knoll (*15*), and others. Until such conditions can be compensated for, it would appear that average seed set would be most descriptive of the species if based upon a reasonably large number of plants. Knowledge of the distribution of these in the range of fertility is also important, however.

Prominent variations in results obtained from year to year would render questionable any effort to classify plants narrowly as to self-fertility. Fluctuations among inflorescences of the same plant and significant differences among clones from the same plant serve to emphasize the difficulty of differentiating between plants. Similarly, small differences between strains might be of questionable significance. Frequent occurrence of zero seed set in one or more bags, where other bagged heads of the same plant may give few to fairly numerous seeds, makes statistical study very difficult if not impracticable.

The studies of the relation of number of inflorescences per bag to seed setting did not indicate any significant differences to exist when one or several heads were enclosed within one covering. This does not support the observations of Frandsen (*4*) or those of Sylven, cited by Nilsson (*23*). However, since the conditions of study and the species observed by Frandsen and Sylven were different from those in the investigation reported here, comparison may not be justified.

[5] Principally by the Division of Nurseries, Soil Conservation Service.

Results of germination tests of seeds from self- and open-pollination are in general agreement with those of Jenkin (9), which indicated selfed seeds to have a somewhat lower germination percentage than those derived from open-pollination. Differences were not consistent, however.

SUMMARY

Results of controlled pollination studies of a number of grass species grown under field and greenhouse conditions are reported.

The following species set seed freely when inflorescences were enclosed: *Agropyron caninum, A. ciliare, A. semicostatum, A. trachycaulum, Bromus carinatus, B. catharticus, B. commutatus, B. madritensis, B. marginatus, B. mollis, B. polyanthus, B. rigidus, B. secalinus, B. tectorum, Elymus canadensis, E. glaucus, E. sibiricus, E. virginicus, Hordeum jubatum, H. murinum, Poa ampla, P. canbyi, P. compressa, P. nevadensis, P. pratensis, Sitanion hystrix, Stipa columbiana, S. comata, S. lettermani, S. robusta,* and *S. viridula.*

The following species were intermediate in seed setting when selfed: *Agropyron elongatum, Festuca elatior, F. elatior* var. *arundinacea, F. idahoensis, F. rubra,* and *Poa juncifolia.* Results for *Hordeum nodosum* and *Secale montanum* were inconclusive.

The following species produced very few seeds when subjected to self-pollination: *Agropyron cristatum, A. dasystachyum, A. desertorum, A. inerme, A. intermedium, A. repens, A. sibiricum, A. smithii, A. spicatum, A. trichophorum, Arrhenatherum elatius, Bromus erectus, B. inermis, Dactylis glomerata, Elymus angustus, E. chinensis, E. condensatus, E. giganteus, E. junceus, E. triticoides, Festuca ovina, Hordeum brevisubulatum, H. bulbosum, Koeleria cristata, Oryzopsis hymenoides, Phalaris arundinacea, Phleum graecum, P. phleoides,* and *Puccinellia distans.*

Field results based upon inflorescences enclosed in bags were generally comparable to those obtained under greenhouse conditions, from isolation of individual plants without bagging.

In most species studied there was considerable variation in both self- and cross-fertility among different plants. Some highly self-sterile species included plants that were sterile also when open-pollinated. Plant variation in seed setting was much greater with self-pollination than with open pollination.

Much random variation occurred in seed setting following self-pollination and open-pollination. Simple explanations of such results were not possible. Interannual fluctuations likewise were great when the same plants were studied in successive years. Classification of plants into narrow groups on the basis of self-fertility appeared to be unwarranted.

Where reliable comparisons were possible, self- and cross-fertility appeared to be positively related in *Agropyron cristatum* and *Bromus inermis*, whereas for *Arrhenatherum elatius* a negative association was indicated.

Differences in seed setting due to the type of paper covering used were generally insignificant. The number of inflorescences enclosed in each bag did not influence the percentage of seeds produced by *Bromus inermis.*

Germination of seeds from self-pollination was slightly inferior to that from open-pollination, though the differences were not consistent.

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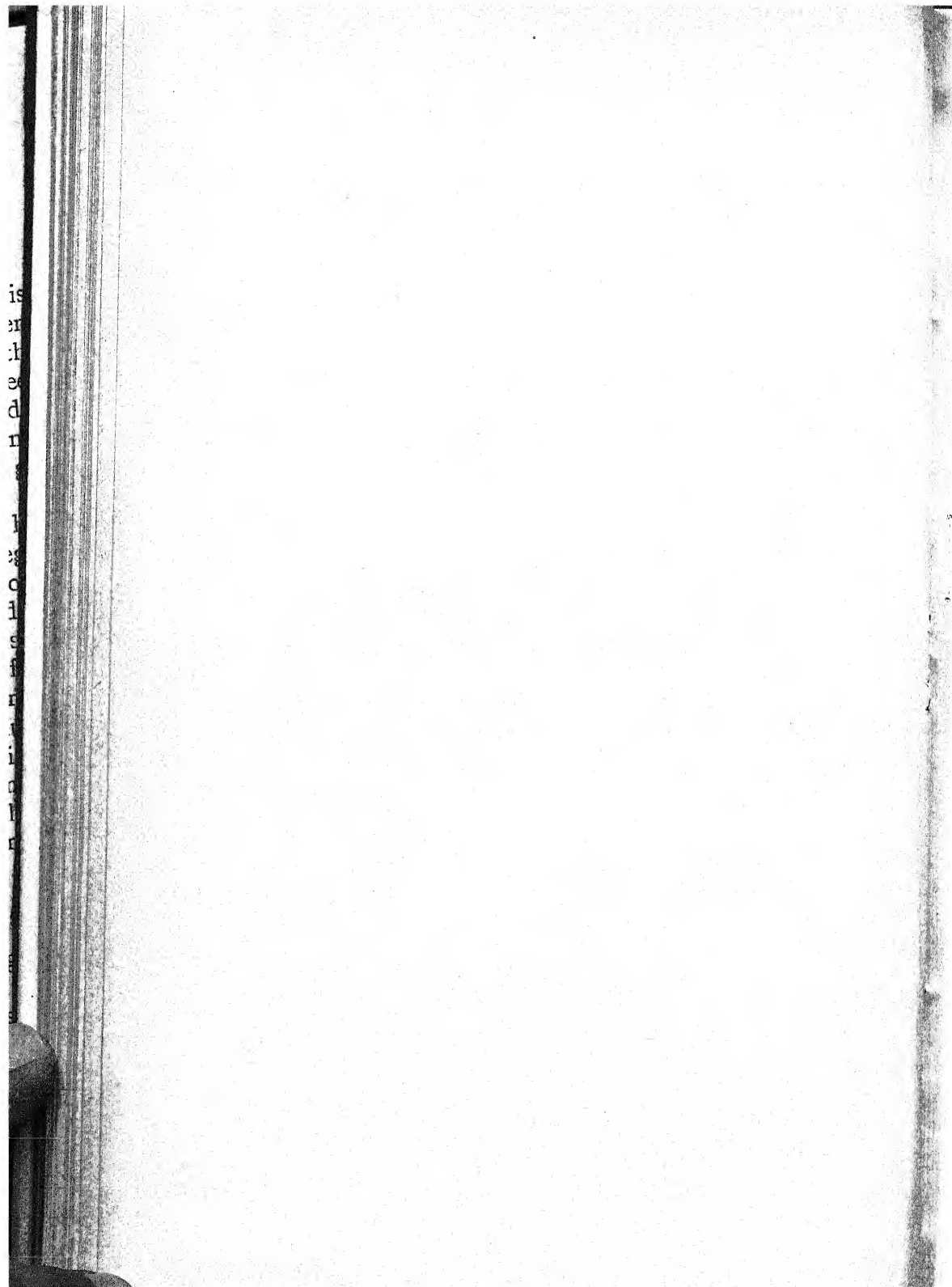
INFLUENCE OF CONTROLLABLE ENVIRONMENTAL CONDITIONS ON REGENERATION OF JACK PINE AND BLACK SPRUCE [1]

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INTRODUCTION

Jack pine (*Pinus banksiana*) and black spruce (*Picea mariana*) are important pulpwood species in the northern Lake States. Knowledge of conditions under which they can be successfully reproduced is essential to their forest management. An experiment was therefore undertaken to test the influences of those environmental conditions which seemed most important in relation to natural reproduction of these species. The influences studied were those of (1) density of tree cover, (2) density of lower ground cover, (3) character of soil surface, and (4) seed-destroying rodents and birds. These are all factors over which man can exercise some control.

In interpreting the results of this experiment, it must be borne in mind that they apply particularly to the shallow soils and relatively cool climate of northeastern Minnesota. Although black spruce occurs both in swamps and on upland, the results pertain only to upland.

REVIEW OF LITERATURE

Many investigations have been made on factors influencing the germination, survival, and growth of conifer seedlings. These studies have in almost all cases dealt with species other than jack pine and black spruce, but they have established several principles that are generally applicable.

Several investigators have shown that soil surface material has a decided influence upon germination and survival of young seedlings. Larsen (*8*)[4] sowed seed of western white pine (*Pinus monticola*) on several kinds of soil surface, including natural duff, ashes, partly burned duff, and freshly loosened soil from which the duff and humus layers had been raked. Germination was decidedly better on the bare loose soil, ashes, and partly burned duff than on the undisturbed duff. The poorer germination on duff as compared with bare soil or ashes was attributed to less favorable moisture conditions. Studies reported by Barr (*1*), Lowdermilk (*12*), Moore (*14*), Shirley (*20*), Griffith (*4*), Robertson,[5] Osborne and Harper (*15*), and LeBarron and

[4] Italic numbers in parentheses refer to Literature Cited, p. 118.

[5] Robertson, W. M. some regeneration problems at the petawawa forest experiment station. Canada Dept. Int., Forest Serv. Res. Note 41, 32 pp., illus. 1935. [Processed.]

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Eyre (*10*) have shown that the surface of the undisturbed forest floor is a poor medium for germination of conifer seeds as compared with exposed mineral soil. Barr showed that seed of Engelmann spruce (*Picea engelmanni*) would germinate freely on humus if watered daily.

Moore (*13*) and Barr (*1*) pointed out that humus differs greatly from mineral soil in moisture-holding properties. Moore (*14*) reported greater seedling mortality on duff than on mineral soil and attributed it in part to insufficiency of moisture which he believed may have resulted from the heavy drain by the mat of plant roots within the humus. According to Barr, drought mortality was no greater on humus than on a mineral soil. Haig (*5*) reported that drought losses were about the same on duff and mineral soil.

Larsen (*8*) observed that seedlings on ashes and mineral soil were taller and sturdier than those on unburned duff in northern Idaho. Moore (*13*) reported more rapid growth of seedlings on well-decomposed humus than on raw humus or mineral soil. Haig (*5*) found better growth on burnt mineral soil than on duff or natural mineral soil.

Heat injury caused by insolation has been shown by Haig (*5*) and Isaac (*6*) to be a major cause of seedling mortality. Haig, working with western white pine and associated trees, found that in full sunlight the surface of duff becomes much hotter than the surface of mineral soil, particularly during May and June, when new tree seedlings are still small and tender. Isaac found that in hot weather in the Pacific Northwest the daily maximum temperatures of an unshaded fire-blackened surface were considerably higher than those of comparable natural-colored mineral soils.

With regard to influence of plant cover on conifer reproduction, Toumey and Neethling (*22*), Haig (*5*), and Isaac (*6*) found that shade reduces or prevents mortality from excessive heat of the surface soil. On the other hand, plant cover competes with the reproduction for soil moisture. Toumey and Kienholz (*23*) demonstrated that trenching of small plots under forest cover is followed by remarkable increases in low vegetation. Korstian and Coile (*7*) determined that during periods of critical dryness significantly greater moisture was available on trenched plots than on adjacent untrenched plots. Shirley (*21*) found that during dry periods soil moisture became critically low more frequently on untrenched than on trenched quadrats. Haig's (*5*) studies with seedlings under tree cover of various densities showed that drought losses were more severe under heavy forest cover than where tree cover was light or lacking. Pearson (*17*), working with ponderosa pine (*Pinus ponderosa*) in the Southwest, concluded that moisture and light relations could not entirely account for the failure of reproduction under trees, and suggested that temperature or some other factor might play a part in it. Shirley (*18*) pointed out that light intensity is usually a governing factor in the growth of vegetation under a forest canpoy, and that (*19*) an understory of shrubs reduces light intensity much more than an overstory of oldgrowth red pine (*P. resinosa*). Shade, by restricting the rise of soil temperature, may retard the seasonal beginning of germination, according to Haig (*5*). Several workers, including Wahlenberg (*27*), Moore (*14*), and Shirley (*20*), have observed that falling leaves may smother small seedlings.

Destruction of seeds and seedlings by birds and rodents is some-times a restrictive factor in the establishment of conifer reproduction (*27, 3*).[6]   Insects, also, may be a cause of loss, as reported by Wahlen-berg (*27*), Pearson (*16*), and Haig (*5*).   Barr (*1*), who grouped bird and insect losses together, observed severe losses of this kind.

Losses of conifer seedlings by disease have been reported in connec-tion with several studies of seedling establishment (*1, 27, 16, 5*). Pearson observed that damping-off losses were less in sunny than in shady locations.   Haig's experiments failed to show consistent differences in damping-off losses between full-sun, part-shade, and full-shade stations; the losses were distinctly greater on duff than on mineral soil except under full shade.   Barr found that losses from fungi were much higher on humus than on clay soil.

EXPERIMENTAL AREA

The site chosen for the experiment is an almost level upland, typi-cally occupied by jack pine and black spruce, on the Superior National Forest near Ely, Minn.   The locality is characterized by a cool and relatively short growing season.   The length of the frost-free period averages 108 days (*24*) at Virginia, Minn., about 45 miles distant but nearest of the long-time weather stations.   Annual precipitation at the same place averages only 26.54 inches (*25*), but is favorably distributed in relation to the summer period.   In the 46 years 1894–1939, monthly precipitation totals during the growing season averaged as follows:

	Inches
May	2. 80
June	4. 05
July	3. 84
August	3. 61
September	3. 71

The soil, of glacial origin, covers the igneous bedrock (gabbro) to a depth of from 18 inches to 3 feet.   It varies greatly in texture. Mechanical analysis of samples taken about 3 inches below the humus layer within the zone of seedling roots showed it to contain 18.9 percent of gravel-sized material.   The finer portion was 65.0 percent sand, 25.0 percent silt, 2.8 percent clay, and 7.2 percent fine clay. Although individual samples had a considerable range, the soil may be described in general terms as a sandy loam.   Its pH value, as deter-mined by the colorimetric method with a LaMotte-Kenney soil-testing kit, was 5.2.

At the time the experiments were inaugurated the timber was 65 to 70 years old.   The dominant jack pines averaged about 65 feet in height, which indicates the site to be of medium quality for the species (*26*).   The timbered area consisted of two separate blocks, approximately one-half mile apart.   On block I jack pine predomi-nated, making up 62 percent of the basal area of the stand, and was followed by black spruce, 16 percent; quaking aspen (*Populus trem-uloides*), 20 percent; and paper birch (*Betula papyrifera*), balsam fir (*Abies balsamea*), and eastern white pine (*Pinus strobus*), 2 percent. On block II black spruce was most prominent, amounting to 42

[6] BURLEIGH, T. D.   THE RELATION OF BIRDS TO THE ESTABLISHMENT OF LONG-LEAF PINE SEEDLINGS IN SOUTHERN MISSISSIPPI.   U. S. Forest Serv., South. Forest Expt. Sta. Occas. Paper 75, 5 pp., illus. 1938. [Processed.]

percent of the basal area of the stand. Jack pine composed 24 percent and quaking aspen 18 percent; the remaining 16 percent was paper birch, balsam fir, eastern white pine, and red pine.

The few shrubs present in the understory, principally American green alder (*Alnus crispa*) and two kinds of blueberry (*Vaccinium angustifolium laevifolium* and *V. canadense*), were small and suppressed. The herbaceous cover differed considerably on the two blocks in both abundance and kind. On block I the principal species were *Aster macrophyllus*, *Cornus canadensis*, *Maianthemum canadense*, and *Rubus pubescens*. The foliage of the herbaceous plants covered half or more of the ground surface, at least during early summer. On block II *C. canadensis* was by far the most common herbaceous flowering plant and there was a scattering of *Aralia nudicaulis*, *R. pubescens*, *Carex* spp., and *Agrostis hiemalis*, but altogether the flowering plants did not cover more than 5 or 10 percent of the ground surface. A hypnum moss, *Calliergon schreberi*, formed an almost continuous carpet over the forest floor on this block.

EXPERIMENTAL DESIGN AND PROCEDURE

The design of the experiment was a split-plot arrangement (*28*). On each of the two timbered blocks 1½ acres were clear cut and an equal area was thinned to 50 percent of the basal area. After the thinning, the light intensity under the tree canopy amounted to 43 percent of full sunlight, according to paired black and white Livingston atmometer spheres 8 inches above the ground surface. In the center of each of the two clear-cut plots and the two thinned plots an area 32 feet by 36 feet was fenced against deer and rabbits. These four areas were designated for detailed seed-sowing tests. As natural seeding of all the experimental plots would be too uncertain and would in any case take too long a time, seed was sown artificially, in a manner resembling natural seeding. The enclosed plots were further subdivided, as shown diagrammatically in figure 1, to bring out the reactions to different factors affecting germination, survival, and growth of reproduction. Specifically, the experiment was designed to answer questions as to the influences of:

1. Density of tree cover:
   a. Half of full density
   b. None
2. Density of lower cover (shrubs and herbs):
   a. Natural
   b. None
3. Character of soil surface:
   a. Undisturbed duff
   b. Burned duff
   c. Scarified surface artificially shaded
   d. Mineral soil
4. Seed-destroying rodents and birds:
   a. Completely excluded
   b. No protection

The treatments were assigned to the various subdivisions by accepted methods of randomization. The lay-out of artificially seeded plots on the 2 blocks, representing in replicate 2 degrees of density of tree cover, 2 degrees of density of low cover, 4 kinds of soil surface, 2 years of sowing, 2 tree species, and 2 degrees of protection from rodents and birds, included altogether 256 units upon which germination and survival could be observed, and an equal number of paired plots to be used in growth determination. To check on natural

seeding, a third series of paired plots was established on each block (fig. 1) and left unsown.

Seeding was carried out in 1937 and 1938. The soil surfaces for the 1937 sowings were prepared during May 1937, and those for the 1938 sowings during September 1937. Burning was accomplished by applying a gasoline brush-burning torch to the natural accumulation of wood and litter upon the ground. This left a layer of humus ½ inch to 1 inch deep, a sprinkling of charcoal, and a very thin deposit
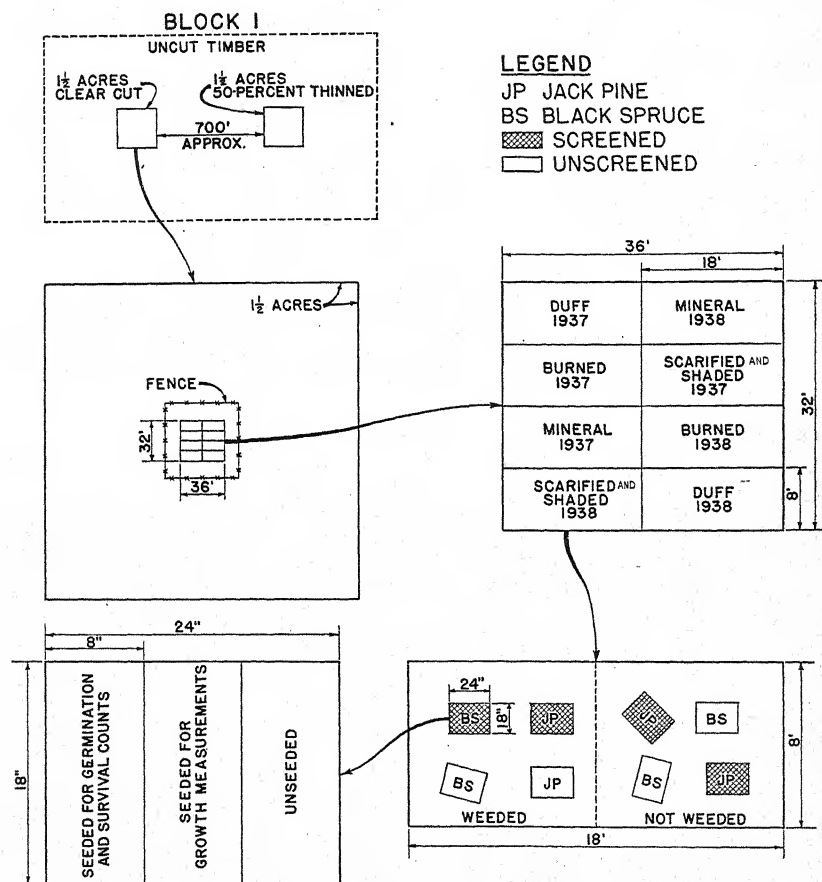
FIGURE 1.—Arrangement of plots in one replication.

of ash, thus closely approximating conditions after a forest fire. Soil acidity tests about 3 weeks later indicated that the pH of the surface material had not been perceptibly affected. Mineral soil surfaces were made ready by stripping away the litter, duff, and humus. Scarified surfaces were prepared by breaking up and mixing the duff, the humus, and a little of the mineral soil. Two layers of sticks were placed crisscross over the scarified surfaces to simulate the shading effect of a light layer of logging slash, concealing approximately 75 percent of the ground surface. The undisturbed duff surfaces re-

quired no preparation. Duff and humus together averaged about 1½ inches thick, varying from less than 1 inch to 4 or 5 inches. The duff was composed of matted conifer needles, leaves, twigs, and mosses in various stages of decay. The whole organic layer was closely interlaced with the roots of trees, shrubs, and herbs.

It was intended that enough seeds be sown to produce about 50 seedlings on each 1-square-foot subdivision. To compensate expected differences in germination, the number of seeds sown per subdivision was varied as follows: Jack pine on mineral, burned, and scarified surfaces, 62; jack pine on duff, 124; black spruce on mineral, burned, and scarified surfaces, 100; black spruce on duff, 200. The seed used in 1938 was from the same lots as that used in 1937. In 1937 the viability of the jack pine seed was 80 percent and that of the black spruce seed was 64 percent; in 1938 the viability of the jack pine seed was still 80 percent, but that of the black spruce seed had dropped to 61 percent.

The 1937 plots were seeded on June 3, 4, and 5, and the 1938 plots in the period May 6–9. The method used was to scratch the surface of the plot lightly with a small stick, scatter the seeds, and pat the surface lightly with the hand to force the seeds into crevices of the loosened surface. This left most of the seeds partly visible except on the duff and the scarified soil, where practically all of them disappeared into crevices. This method may have brought the seeds into slightly closer contact with the soil than natural seeding would have done, but was necessary to prevent excessive washing away of seeds during heavy spring rains.

Screen boxes to give protection from rodents and birds were put in place on half the plots immediately after sowing and left until the end of the first growing season. The type of screen used reduced light intensity by only 10 percent as determined with Shirley's light meter.

Records were taken on block I of maximum and minimum air temperatures, soil surface temperatures, precipitation, evaporation, light intensity, and soil moisture content at various depths. On block II, such measurements were limited to precipitation and soil moisture. Precipitation was measured with rain gages similar to the standard instrument, two being used on the cleared areas and three on the plots having tree cover. Daily maximum temperatures on the four kinds of soil surface were measured on both kinds of cutting, with eight small maximum thermometers, and a continuous record of temperature on the mineral surfaces was kept with two thermographs. Soil moisture was determined partly by the oven-drying method and partly with porous pot tensiometers. Evaporation and light intensity were measured with paired black and white Livingston atmometer spheres, six to eight pairs being used at a time.

Germination and survival counts were started soon after germination began. While the seedlings were small and tender they were counted every 2 or 3 days, but as they increased in size the interval was gradually extended to about 30 days in the third growing season. The small amount of germination that occurred in the second growing season was excluded from the analysis on the assumption that seedlings getting such a late start were of little practical significance. Growth was recorded in terms of height of tops at end of each growing season and, beginning with the second year, in terms of green weight

of tops at end of growing season.   To ascertain depth of root penetration, seedlings were dug up at intervals of 7 to 10 days during the growing season of 1937.

GERMINATION

Germination was prompt.   In the 1937 series, it began on June 10, 7 days after the seed was sown, and was largely completed by June
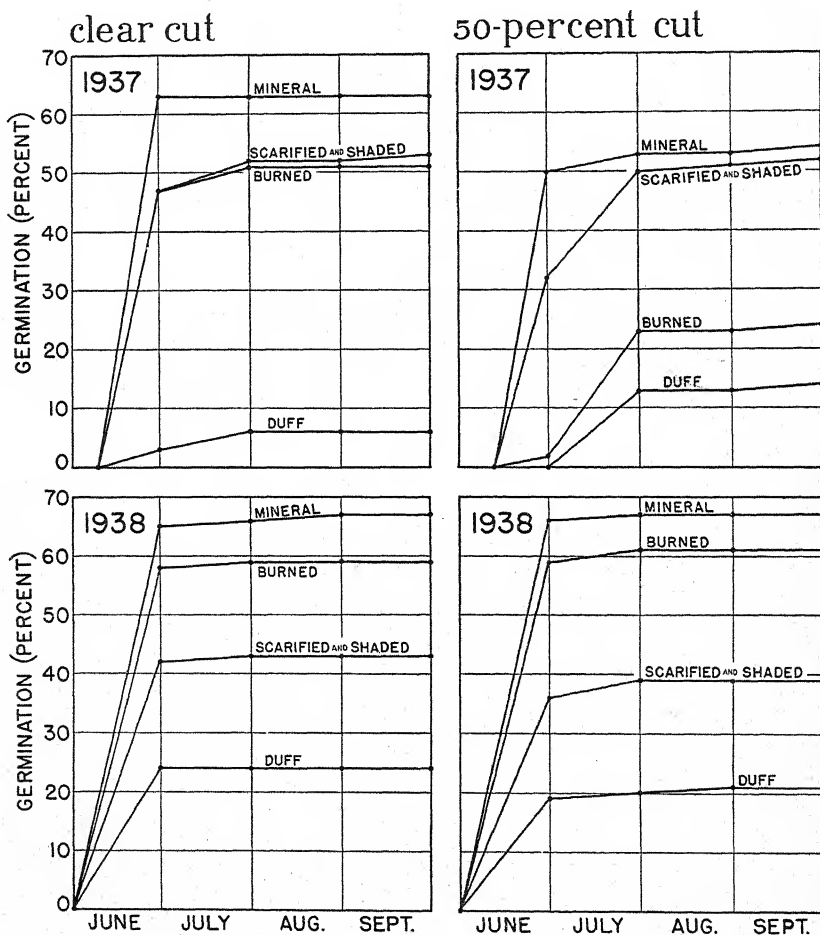
FIGURE 2.—Course of germination of jack pine under various conditions as to soil surface and tree cover in 1937 and 1938.

30.   There was, however, some activity during July, and scattered seedlings appeared during August and September (figs. 2 and 3). Jack pine germinated somewhat more promptly than black spruce. In the 1938 series, germination began on May 30, 24 days after sowing, and was practically completed by the end of June.

The somewhat earlier and more complete germination in 1938 was apparently the result not only of earlier sowing but also of more

abundant precipitation during June of that year (table 1). The longest period in June without at least a trace of precipitation was 3 days, consequently germination progressed rapidly. The heavy precipitation in July 1937 (table 1) gave seeds a further opportunity to germinate, but in July 1938 rainfall was only about half of normal and germination stopped abruptly. Both species germinated promptly whenever temperature and moisture conditions were favorable. This
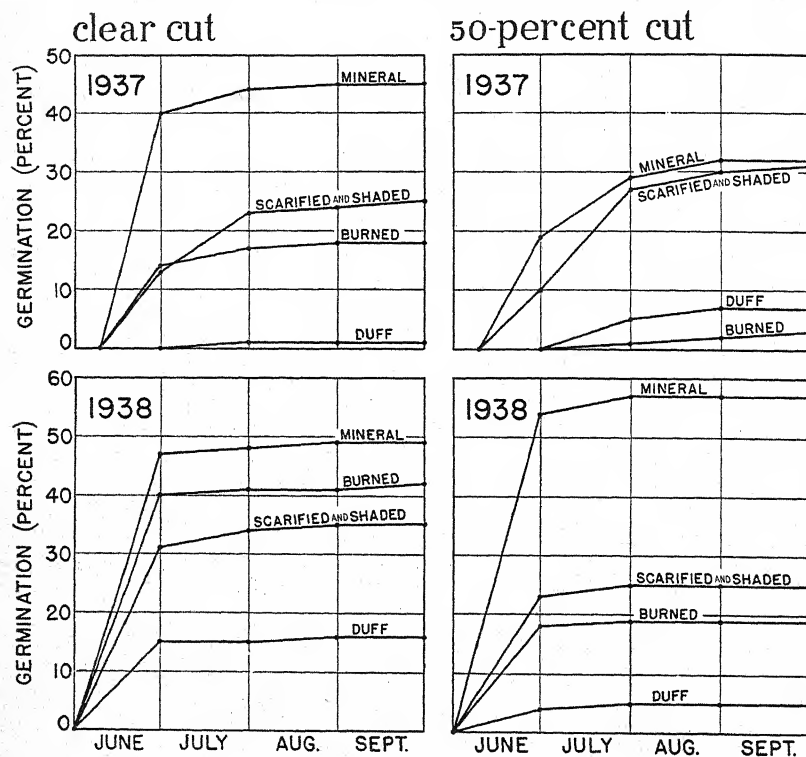
FIGURE 3.—Course of germination of black spruce under various conditions as to soil surface and tree cover in 1937 and 1938.

observation may be of considerable practical importance in planning natural-regeneration operations, especially because of the peculiar seeding habits of both jack pine and black spruce. In the case of jack pine, release of seed by the serotinous cones can be brought about by lopping and scattering the slash after logging (*11*). The peculiarity of black spruce seed dissemination is that after the seed has ripened it is dispersed slowly and continuously over a period of 2 or 3 years (*9*). It is likely, therefore, that some recently escaped black spruce seed will always be present when the timber is cut, regardless of time of year.

TABLE 1.—*Monthly precipitation on study area during growing seasons* [1]

Month	1937	1938	1939
	Inches	*Inches*	*Inches*
May_____	3.72	3.51	4.70
June_____	1.11	3.66	4.44
July_____	5.03	2.00	2.24
August_____	4.69	3.12	6.76
September_____	3.68	1.71	3.85

[1] Rain gage was located on clear cutting, block I

Although germination was prompt, it was only moderately complete (table 2). The overall germination of jack pine (44 percent) was considerably better than that of black spruce (26 percent); this is largely accounted for by the difference between the initial viability percentages of the two species, 80 and 62.5.

TABLE 2.—*Average germination in relation to soil surface, 1937 and 1938*

Soil surface	Jack pine	Black spruce	Average
	Percent	*Percent*	*Percent*
Mineral_____	63	46	54
Burned_____	49	20	35
Scarified and shaded__	47	29	38
Duff_____	17	7	12
Average_____	44	26	35

So little germination of naturally disseminated seeds took place on the unsown plots as to make it appear certain that natural seeding had not materially increased stocking on the artificially seeded plots.

Germination varied strikingly with soil surface, but both jack pine and black spruce reacted quite consistently to the different soil conditions, as shown by the averages of both years in table 2. Mineral soil proved to be decidedly the most favorable medium and duff the poorest for both species, for both years of sowing, and on both clear-cut and 50-percent-cut areas (figs. 2 and 3). The comparative responses on burned duff and scarified-shaded duff were less consistent. In 1937, when precipitation during June was deficient, scarified-shaded duff made the better showing, but in 1938, when June rainfall was heavy, better germination occurred on the burned duff. Because the layers of crossed sticks retarded evaporation, by reducing air movement and lowering temperature, scarified-shaded duff was almost as favorable a medium as mineral soil in 1937, but had a superabundance of moisture in 1938.

The growing seasons of 1937 and 1938 by no means represent the extremes of dryness and wetness for northeastern Minnesota, but the differences were sufficient to give an idea of the influence of rainfall distribution during the germination period.

The germination responses to the different media correspond to the moisture-holding characteristics and textures of the media. Mineral soil offered the least resistance to the transfer of water to the seeds and undisturbed duff the most. The wilting coefficients

(as determined by the standard centrifuging method) of the mineral soil, scarified duff, burned duff, and undisturbed duff were 14, 54, 56, and 77 percent, respectively. Furthermore, mineral soil has the finest texture, which permits closer contact with the seeds and thus facilitates the transfer of moisture. The ashes and bits of charcoal in burned duff and the admixture of mineral soil in scarified duff made these materials intermediate to mineral soil and undisturbed duff in respect to wilting coefficient and texture.

Protective screening against seed-eating birds and rodents had a rather surprisingly small effect on germination. On screened and unscreened plots, respectively, germination percent averaged 48 and 40 for jack pine, 27 and 24 for black spruce. The effort to find out the effect of seed destruction by birds and rodents resulted less conclusively than any other phase of the experiment, probably because the interval between sowing and germination did not give seed eaters the normal opportunity to locate the seed. Also, the population and food habits of birds and rodents may vary considerably from year to year, and during the time of the experiment these variations may have been in a favorable phase.

The influence of tree cover and that of low cover on germination were not of practical consequence. They were nearly the same for both species.

Altogether, the results of germination counts indicate that (1) shade, as from crossed sticks (logging slash), is beneficial to germination in dry seasons; (2) germination on duff, both natural and burned, is better in wet seasons than in dry seasons; (3) mineral soil is the most consistently reliable medium for germination among the four studied; and (4) undisturbed duff is the poorest.

SURVIVAL

Seedlings began to die within a few days after the first ones appeared. Survival averaged 75 percent at the end of June, 56 percent at the end of July, and 42 percent at the end of August; but from August 31 of the first growing season to September 30 of the second only 4 percent of the original number of seedlings died, leaving a net survival of 38 percent. Although total mortality varied considerably between species, and greatly between the two degrees of tree cover and among the four kinds of soil surface, the time of year when mortality occurred was practically uniform (figs. 4 and 5). The loss during the third growing season (data from 1937 series only) was less than 1 percent. It is evident that the first 3 months after germination are the most critical period in seedling establishment.

As would be expected, jack pine materially exceeded the smaller black spruce in capacity to survive, survivals at the end of the second growing season for the pine and the spruce being 54 percent and 22 percent, respectively. This suggests that regeneration of black spruce requires either greater amounts of seed or more favorable environmental conditions then equally successful regeneration of jack pine. In general, survival of both species followed the same trends in relation to the various environmental conditions; a condition harmful to one species was usually harmful to the other.

Over-all survival of both species during the first 2 years varied most strikingly with soil surface (figs. 4 and 5), averaging 65 per-
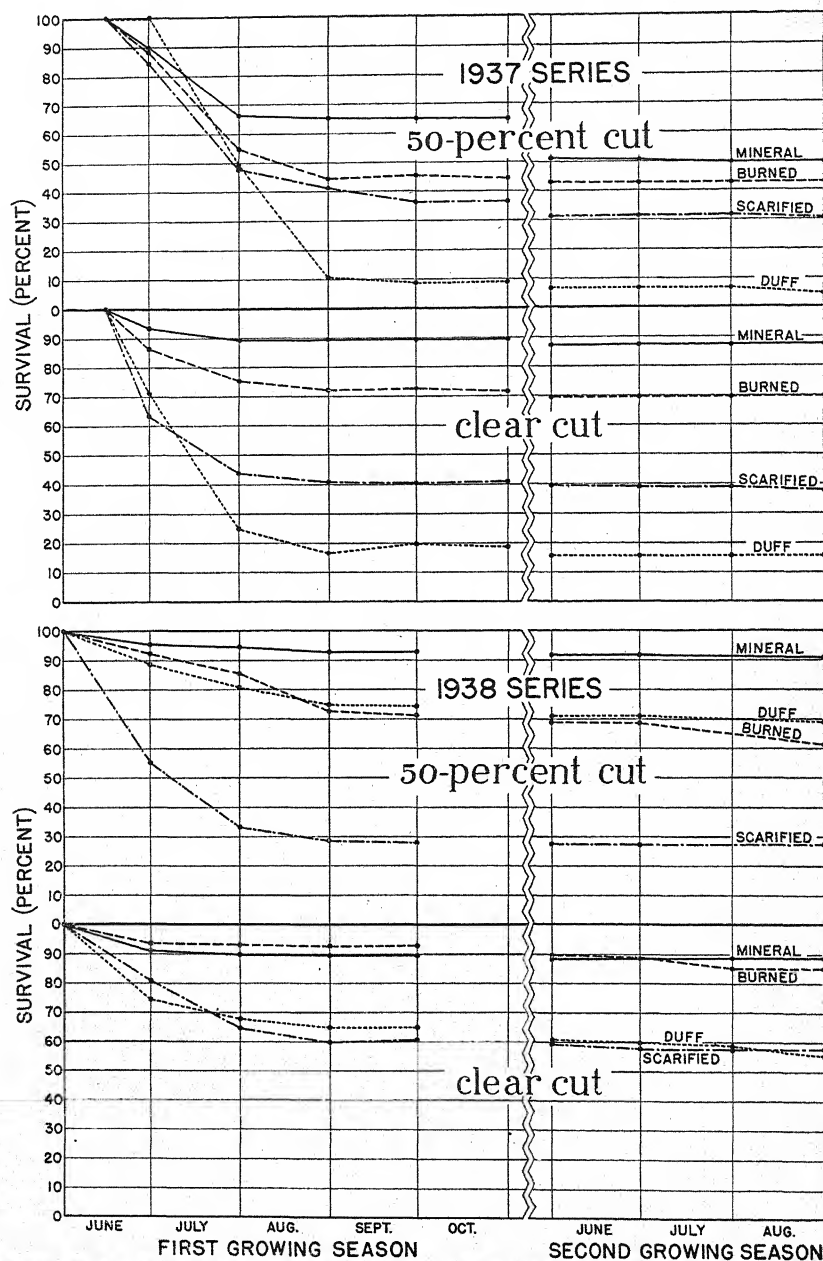
FIGURE 4.—Survival during first and second growing seasons of jack pine seedlings on various soil surfaces and on areas clear cut and 50-percent cut.
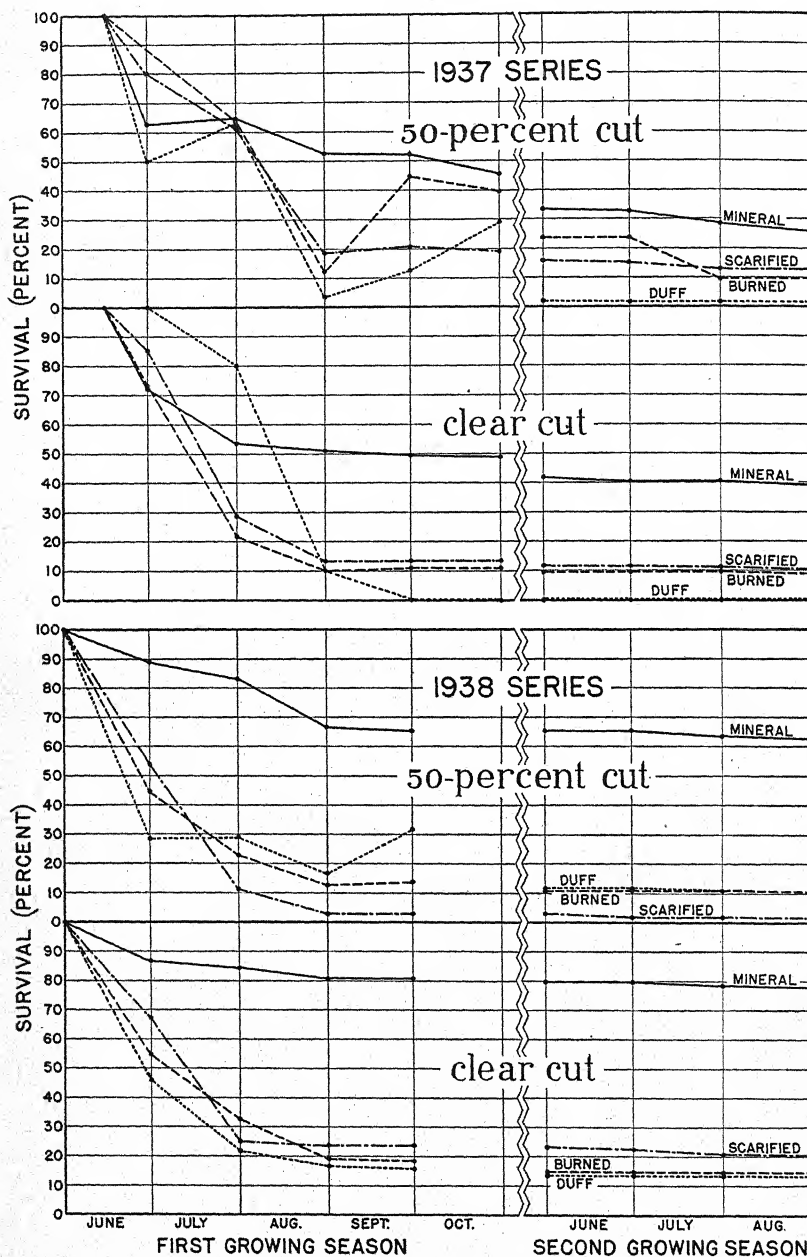
FIGURE 5.—Survival during first and second growing seasons of black spruce seedlings on various soil surfaces and on areas clear cut and 50-percent cut.

cent on mineral soil, 37 percent on burned duff, 25 percent on scarified and shaded soil, and 24 percent on undisturbed duff. The advantage of mineral soil with regard to survival, as well as to germination, makes this kind of surface outstandingly superior for seedling establishment. The chance that a seed will germinate and the seedling survive 2 years is 12 times as great on mineral soil as on undisturbed duff. It may be noted that methods for exposing the mineral soil with power machinery as a practical silvicultural measure are already well developed (*2*).

Tree cover had considerable influence, the percentages for clear-cut and partially cut plots being 45 and 32, respectively. There was a great difference in 2-year survival between the two annual sowings, the average for the 1937 series being 30 percent and that for the 1938 series 46. The better showing of the 1938 series apparently resulted from the earlier germination (figs. 2 and 3) and the heavier rainfall during June (table 1).

CAUSES OF MORTALITY

The relations of soil surface, tree cover, and low cover to seedling survival assume a more significant aspect when the specific causes of mortality are considered, because damage by each of the chief destructive agents, both biotic (insects and damping-off fungi) and physical (heat and drought), was apparently affected by one or more of the controlled conditions. The recorded causes of death and frequency of each are as follows:

	Percent		Percent
Insects	52	Mildew	2
Damping-off fungi	17	Root rot	1
Heat	10	Plant competition	(trace)
Drought	8	Miscellaneous	3
Frost heave	3	Unknown	4

A good many seedlings were killed by combinations of factors, and sometimes no cause could be identified with certainty. However, these uncertainties were relatively infrequent and do not affect the principal conclusions. To a considerable extent the main causes of mortality—insects, damping-off fungi, heat, and drought—operated concurrently, and hence "competed" among themselves. Particularly if there had not been such exceedingly high insect losses, the other agents probably would have disposed of more seedlings.

The distribution of losses due to each cause with regard to species, tree cover, and soil surface, in percent of total germination, is shown in table 3.

TABLE 3.—*Mortality* [1] *(percent) during first 2 years, by species and cause, in relation to tree cover and soil surface*

Species, tree cover, and soil surface	Insects	Damping-off	Heat	Drought	Frost heave	Mildew	Root rot	Competi-tion	Miscellane-ous	Unknown	Total
Jack pine: Clear cut:											
Mineral	5.9	2.0	0	0.5	0.4	0	0.4	0.1	1.8	0.4	11.5
Burned	8.7	6.3	.6	1.3	.2	2.2	.1	2.0	0	1.2	22.6
Scarified	39.7	5.4	.2	1.5	0	1.2	.6	.2	.2	3.4	52.4
Duff	37.7	5.2	3.0	3.7	0	1.6	.6	2.0	3.2	7.2	64.2
Average	23.0	4.7	1.0	1.8	.2	1.2	.4	1.1	1.3	3.0	37.7
50-percent cut:											
Mineral	7.8	4.2	.1	8.4	7.3	0	.3	0	.6	1.3	30.0
Burned	17.2	14.7	5.0	7.1	0	1.2	2.0	.6	4.4	1.0	53.2
Scarified	50.0	12.4	.6	4.1	.1	1.1	.6	0	2.3	1.4	72.6
Duff	38.1	14.6	3.0	4.4	0	2.3	0	0	1.1	.4	63.9
Average	28.3	11.5	2.2	6.0	1.8	1.1	.7	.2	2.1	1.0	54.9
Average jack pine	25.6	8.1	1.6	3.9	1.0	1.2	.6	.6	1.7	2.0	46.3
Black spruce: Clear cut:											
Mineral	17.3	8.7	1.9	3.1	4.0	.2	.8	0	2.2	3.1	41.3
Burned	36.1	17.7	24.6	4.3	0	.8	1.7	.4	2.3	2.1	90.0
Scarified	67.1	11.0	.6	1.0	0	.8	.8	0	1.2	1.6	84.1
Duff	44.6	9.8	22.6	5.0	0	.1	.4	0	.6	0	83.1
Average	41.3	11.8	12.4	3.3	1.0	.5	.9	.1	1.6	1.7	74.6
50-percent cut:											
Mineral	19.7	3.1	.4	17.6	9.3	0	1.2	0	2.4	2.4	56.1
Burned	25.2	13.9	27.4	3.9	0	10.4	.1	0	6.2	2.8	89.9
Scarified	61.5	22.7	.3	5.0	.3	.8	.4	.1	.6	1.2	92.9
Duff	54.9	14.7	5.4	3.8	3.1	.8	.4	0	.4	8.5	92.0
Average	40.3	13.6	8.4	7.6	3.2	3.0	.5	0	2.4	3.7	82.7
Average black spruce	40.8	12.7	10.4	5.5	2.1	1.7	.7	.1	2.0	2.7	78.7
Average both species	33.2	10.4	6.0	4.7	1.5	1.5	.6	.3	1.8	2.4	62.4

[1] In terms of total germination.

INSECTS

Insects, causing half the mortality, were by far the most important source of seedling losses. Insects actually observed eating the tops of young seedlings were a species of grasshopper (*Camnula pellucida*), some very small caterpillars (*Halisidota* spp.), the larvae of a sawfly (*Empria* spp.),[7] and the spruce budworm (*Cacoecia fumiferana*). One small larva, not identified, ate 17 spruce seedlings on 1 plot within 11 days. A few seedlings were killed by larvae of June beetles (*Phyllophaga* spp.).

Loss of tops and cotyledons through insect activity began immediately after the first seedlings appeared, and continued through July and August. At first it was thought that birds might be causing some of the loss, but frequent observations and the similarity of damage on screened and unscreened plots led to the conclusion that insect activity was the sole cause. The total damage attributed to insects was practically equal on screened and unscreened plots.

[7] The three insects just named were identified by A. G. Ruggles, State entomologist, Minnesota Department of Agriculture, Dairy, and Food.

Insects are recorded to have killed approximately 26 percent of all the jack pine that germinated and 41 percent of the black spruce, but since some seedlings may have been killed by other agents before the insects had a chance at them, the reported values (table 3) may not show the full possible effect of insect damage.

Insect-caused mortality was affected very little by tree cover, and was not appreciably greater on unweeded than on weeded plots; but it varied to a surprising extent with soil surface. Losses were greatest on the scarified-shaded surfaces, next greatest on undisturbed duff, less on the burn, and least on mineral soil. These differences were large and fairly consistent for both species, both degrees of tree cover, and both degrees of low cover.

Why should the nature of the soil surface affect insect damage? There appear to be several possible explanations. With the exception of grasshoppers, all the insects observed feeding were small larvae having little ability to travel. Probably these insects had wintered (either as eggs or as larvae) very close to where they attacked the seedlings. Overwintering larvae or eggs would of course be destroyed by burning the duff or removed in the process of preparing the mineral soil surface. This could easily account for the light damage on the burned duff and mineral soil and offers an additional explanation for the fact that reproduction is frequently successful after forest fires. If insects migrated to the plots after the surfaces were prepared, they may have preferred the irregularity and loose structure of the scarified soil and the undisturbed duff. Neither of the explanations suggested would account for the greater losses on scarified-shaded soil as compared with undisturbed duff. Possibly the sticks laid over the scarified surfaces provided some element of comfort or protection that encouraged the insects to concentrate or permitted them greater activity.

The fact that insects killed about 60 percent more black spruce than jack pine may indicate a species preference. More probably it was due to the smaller size of the spruce seedlings, because of which they were easier to kill and a larger number were required to provide an equal quantity of food.

The most likely reason why low vegetation had little or no effect on seedling damage by insects is the fact that during the early part of the first growing season the low vegetation was rather sparse, owing to the recent soil surface preparations, and could hardly exert a strong influence until much of the insect damage had been done.

DAMPING-OFF FUNGI

Damping-off fungi, the second most important cause of mortality, killed somewhat more black spruce than jack pine. Losses were generally lowest on mineral soil. They were greater under tree cover than in the clearings, particularly among jack pine seedlings, but the data are too erratic to justify drawing conclusions.

HEAT

Although heat caused only 10 percent of the total mortality, it gave evidence of being potentially a very serious source of seedling injury. Losses on mineral soil and on scarified soil were consistently so small

as to be negligible, but those on undisturbed duff and on burned duff, particularly the latter, were considerably higher. Temperature measurements explain this. Daily maximum temperatures on the undisturbed duff and burned duff surfaces frequently exceeded 120° F. and occasionally exceeded 140°. The highest temperatures observed on undisturbed duff and burned duff during both seasons were, respectively, 153° and 159°. On the other hand, the temperatures of mineral soil and scarified-shaded soil seldom went above 100°.

The greatest number of low daily maximum temperatures occurred on the scarified-shaded soil, obviously as a result of the heavy shade cast by the two layers of sticks. The relatively low temperatures on the mineral soil are largely accounted for by the cooling effect of evaporation. Conversely, the relatively high temperatures on the undisturbed duff may have been due to the dryness of this material, which permitted little such cooling. The dark color of the burned duff may explain in part the relatively high temperatures on that surface. Contrasts in color of the various materials, however, were not nearly so striking as that described in a report by Isaac (6) on a seedling-establishment study. Here the mineral soil was a yellowish brown, the undisturbed duff was a light grayish brown, and the burned duff was a mixture of black and grayish brown. The differences in color were apparently not great enough to cause very much variation in temperature.

As would be expected, the tiny black spruce seedlings proved decidedly more sensitive to heat than the relatively large jack pines.

Observed losses due to heat were too few in number to justify definite conclusions. Heat losses were practically the same under the 50-percent tree cover as on the cleared areas. This is not surprising, for measurements showed that daily maximum surface temperatures were almost as high under tree cover as in the clearings. Tree shadows passing over the bulb of the soil thermograph caused very rapid fluctuations in the temperature record, but during shadow-free periods the temperatures recorded on the timbered and clear-cut areas were practically the same. Although the succession of shadows considerably shortened each daily exposure to lethal temperatures, this did not affect survival. In seasons having longer periods of hot weather the results would probably be different. Failure of the partial tree cover to restrict maximum surface temperatures greatly can be accounted for only by the somewhat drier condition of the surface soil under the trees.

DROUGHT

Drought, like heat, was decidedly not a limiting factor in seedling establishment in this study. It caused only 8 percent of the total deaths. The fact that the July 1938 rainfall of only 2 inches (approximately half of normal) was sufficient for most of the seedlings suggests that drought is not a common limiting factor in northeastern Minnesota. Although the losses were too light to provide decisive evidence, they are suggestive of the effect of drought in critically dry years. Most drought losses occurred soon after germination. Mortality due to drought was higher in spruce than in pine, and higher under tree cover than on the clear-cut areas (table 3). It did not vary consistently with soil surface. It was higher on unweeded plots than on weeded plots.

The indication that soil surface material does not greatly influence drought losses is corroborated by data taken by Barr (*1*) showing that spruce seedlings endured an artificially maintained drought as long in humus soil from a spruce forest as in clay soil and sand soil.

Soil moisture determinations made by the oven-drying method during the 1937 growing season indicated that moisture was generally present in quantities adequate for survival. The moisture content tended to be a little higher on the clear-cut areas than on the areas having tree cover, although the differences were so small as to appear inconsequential.

The study of drought mortality, which included the digging of seedlings at frequent intervals to determine extent of root penetration, failed to bring out any consistent relation between progress of the season and drought resistance of the seedlings. Several reasons for this may be cited: (1) the frequent summer rains typical of northeastern Minnesota prevented establishment of anything like a regular moisture gradient in the soil. (2) The several horizons (including duff and humus) in the upper 3 inches of soil varied so greatly in texture, wilting coefficient, and thickness that it was most difficult to determine whether moisture was available during critical periods. (3) Even within relatively small samples (50 to 75 gm. dry weight) of organic soils that show little or no available moisture, there are likely to be fractions, such as small dead roots and decayed wood, from which seedlings can extract water. Seedling rootlets showed a decided tendency to follow the inside surfaces of dead roots and to grow into rotten wood. (4) Because scattered seedlings continued to appear throughout the summer and, in 1937, through early autumn, the seedlings were never at a uniform stage of development, and so presumably were never uniform in drought resistance.

The black spruce seedlings extended their primary rootlets downward about 0.5 inch, and thus gained some protection against superficial drying of the soil, within a week after germination. Additional downward growth, however, was slow, and at the end of the first growing season few seedlings had penetrated to a depth greater than 2.5 inches. Most of the roots developed in horizontal planes, and only a few entered mineral soil except on the plots where the mineral soil was exposed. The primary roots of jack pine penetrated more rapidly and to a considerably greater depth than those of black spruce. During the first 10 days they extended downward from 1.4 to 1.8 inches, and by the end of the first growing season they had reached depths of 4 to 7 inches. Although a considerably greater proportion of the jack pine than of the black spruce roots grew down into the mineral soil, the jack pine formed an extensive system of lateral roots in the humus soil on the burned, scarified, and undisturbed duff surfaces.

From the observations made on root development, it is evident that an extended drought causing the soil to dry out to a depth of 3 inches would probably result in an almost complete loss of 1- and 2-year black spruce seedlings. Jack pine is probably less subject to killing by drought than black spruce, because of its deeper-root penetration.

Frost Heave

Frost-heave losses occurred almost exclusively on mineral soil, and more commonly under tree cover than on the clear-cut plots (table 3).

The losses were greater in ratio to the total number of seedlings exposed than the tabulated data indicate, because many seedlings died from other causes before frost heave occurred. This is especially true of black spruce under tree cover. The longer roots of jack pine account for its lighter mortality. Frost heave disturbed trees much more commonly than it actually killed them. Many seedlings lifted almost free of the soil nevertheless managed to survive.

OTHER CAUSES

Early spring examination at the beginning of the second growing season disclosed that a small number of seedlings of both species had been killed apparently by fungus organisms, which had formed mats of mycelia over the needles and buds. The roots of these seedlings were quite sound and strong, indicating that they had not died until after the tops were killed. These losses, attributed in table 3 to mildew, were confined almost entirely to the three organic surfaces. For the most part the affected seedlings were the smaller and weaker ones on the unweeded plots. Losses attributed to root rot were caused by some unidentified factor that killed the seedling roots of both species on all four kinds of soil surface, but chiefly among the smallest and weakest seedlings on the unweeded plots.

The few seedlings classified as killed by plant competition were small, suppressed, and severely crowded.

"Miscellaneous" causes, to which a very small part of the mortality was attributed, included flooding, washing, failure of radicle to enter the soil after the seed germinated on the surface, abnormal germination, mechanical injury by falling sticks, trampling by deer, and smothering by leaves. The fences around the plots afforded almost complete protection against deer, snowshoe hares, and woodchucks.

TOP GROWTH

The most contrasting results in this experiment occurred in the phase concerned with top growth (fig. 6). The growth of seedlings was observed under almost optimum conditions, and under conditions in which life could barely be sustained. The most notable feature of the growth phase was the excellent response of both species to complete removal of tree cover and low cover.

HEIGHT OF 1-YEAR-OLD SEEDLINGS

The average height of 1-year-old jack pine seedlings on the clear-cut plots was 2.4 inches, as compared with 1.4 inches on the partial cuttings. On the weeded plots seedlings averaged in the first year 2.1 inches, and on the unweeded plots 1.7 inches. On clear-cut weeded plots the average was 2.7 inches, as compared with 1.3 inches on partially cut, unweeded plots. The better growth on clear-cut weeded plots was probably due to more favorable moisture, light, and temperature conditions in the open. Under heavy herbaceous vegetation, light intensities were undoubtedly much lower than the 43 percent recorded under the 50-percent tree cover.

Jack pine seedlings on the scarified-shaded plots averaged 1.5 inches in contrast to approximately 2 inches for those on the burned duff, natural duff, and mineral soil. The difference was clearly due to the unfavorable shading effect of the crisscrossed sticks. Once the seedlings had developed to a fair height above the sticks, they grew as

rapidly as any others.   The small size of these seedlings probably made them more susceptible to various agents of injury.   The 1938 series averaged 40 percent greater in height at the end of the first growing season than the 1937 series, owing, no doubt, to the earlier date of sowing in 1938, which lengthened the growing season by 2 to 3 weeks.
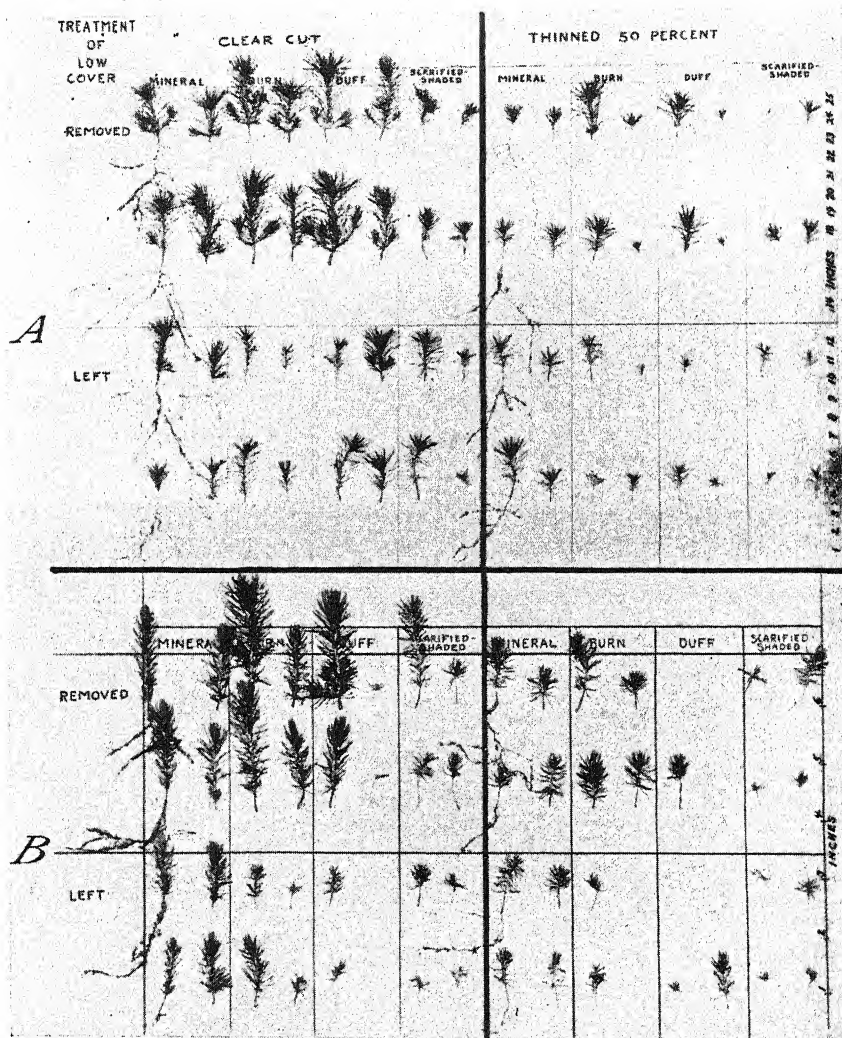
FIGURE 6.—Representative seedlings (*A*) of jack pine and (*B*) black spruce, illustrating comparative development during first growing season (1938) under various conditions as to tree cover, low cover, and soil surface.   The two species are shown on different scales, to permit inclusion in the same figure.

Spruce followed growth trends similar to those of jack pine, although its growth was not nearly so great.   For instance, heights of 1-year-old seedlings averaged 1.0 inch on clear-cut plots and 0.6 inch in the partial cuttings.   It would appear safe to infer that the same factors affected growth of both species in about the same degree.

WEIGHT AND HEIGHT AT 2 YEARS

By the end of the second season, the jack pine seedlings growing under the more favorable conditions had far outstripped the others in
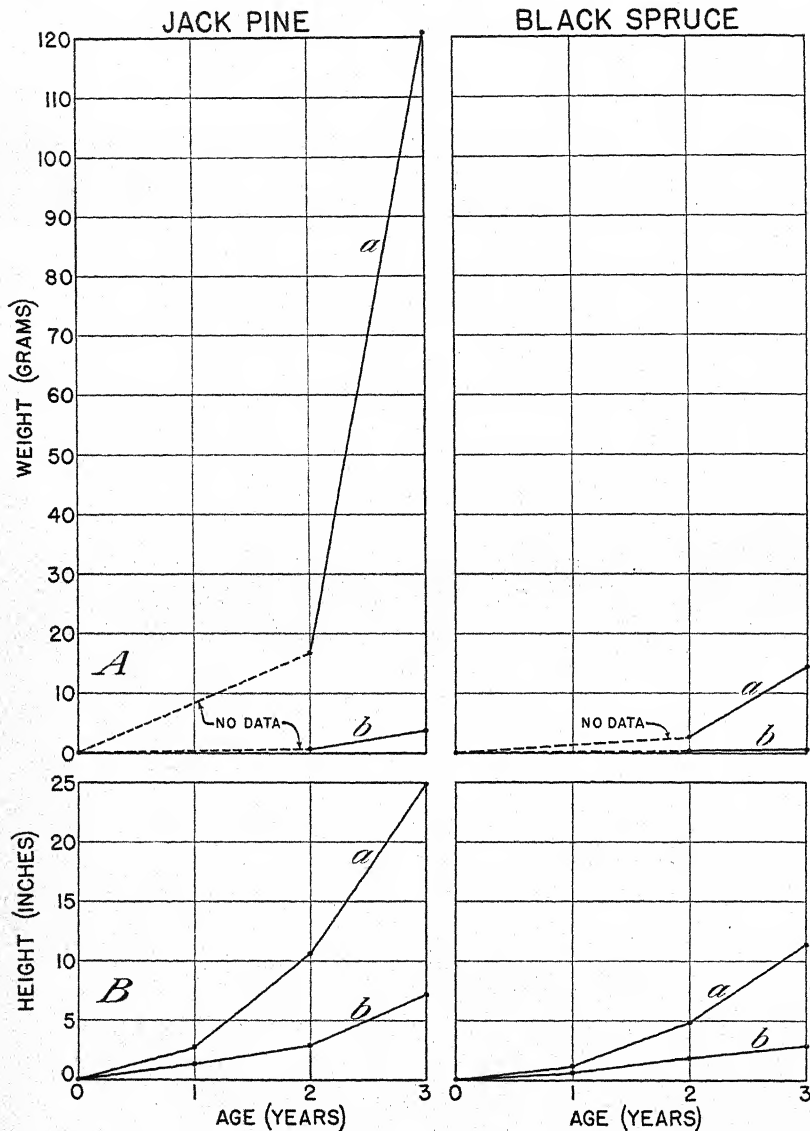
FIGURE 7.—(*A*) Average green weight of tops and (*B*) average height of tops of jack pine and black spruce on (*a*) clear-cut weeded plots and (*b*) 50-percent-cut unweeded plots.

weight and height (fig. 7, table 4).   Clear cutting and weeding both proved beneficial independently, and the two treatments combined gave outstandingly good results.   Weeding resulted in a great advan-

tage in weight on all four kinds of soil surface, but especially on the burned and the undisturbed duff.   Weight of tops at the end of the second growing season did not vary appreciably with year of sowing.

TABLE 4.—*Average green weight of tops of 2-year-old seedlings in relation to low cover, tree cover, and soil surface*

Tree cover and surface condition	Jack pine			Black spruce		
	Weeded	Not weeded	Average	Weeded	Not weeded	Average
Tree cover:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Clear cut	16.82	2.60	9.71	2.49	0.22	1.36
50-percent cut	3.51	.55	2.03	.23	.14	.18
Average	10.16	1.58	5.87	1.36	.18	.77
Soil surface:						
Mineral	8.28	2.52	5.40	.96	.24	.60
Burned	13.72	1.19	7.46	1.81	.16	.98
Scarified and shaded	8.23	1.09	4.66	.52	.12	.32
Duff	13.15	1.46	7.30	4.15	.10	2.12
Average	10.84	1.56	6.20	1.86	.15	1.00

The green weight of the tops of 2-year-old seedlings was much less for black spruce than for jack pine, but the relative responses to tree cover, low cover, and soil surface were very similar (table 4, fig. 7). For black spruce, as for jack pine, the combined effect of absence of tree cover and absence of low cover was much greater than the effect of either of these conditions alone.

Both jack pine and black spruce seedlings growing on mineral soil were appreciably less tall at 2 years than those growing on undisturbed duff and burned duff.   It is believed that this was due to a deficiency in some nutrient, probably nitrogen, caused by the removal of the humus, since the seedlings on the organic surfaces had a darker green color from the time they were a few weeks old.

HEIGHT AND WEIGHT AT 3 YEARS

At 3 years, jack pine excelled black spruce in height and weight of tops by a greatly increased margin (fig. 7).   The tops of jack pine seedlings on the clear-cut and weeded plots averaged 25 inches in height and 121 gm. in weight; the corresponding values for black spruce tops were 12 inches and 14 gm.   The tallest individual 3-year-old jack pine seedling observed was 32 inches high, and the heaviest one weighed 343 gm.   These were free-grown seedlings from clear-cut, weeded plots having undistrubed duff surfaces.   The largest single 3-year-old black spruce seedling was 18 inches tall and weighed 77 gm. It grew on a clear-cut, weeded plot having a burned surface.

On unweeded plots in the 50-percent cuttings, the weights of 3-year-old jack pine and black spruce seedlings averaged only 3.8 gm. and 0.2 gm., respectively.   Obviously, such meager seedlings are unthrifty and have little chance of success.

CONCLUSIONS

The results of the experiment reported herein indicate that:

(1) Seedlings of jack pine and black spruce respond much the same to the chief environmental factors, although the jack pine seedlings,

being larger and faster growing, have a somewhat better chance of survival and establishment. In fact, the similiarity of the responses of the two species, in germination, survival, and growth, confirms the finding in an earlier study (*9*) that black spruce is capable of functioning as a fire species in northern Minnesota in much the same manner as jack pine.

(2) For these species bare mineral soil is the best of the four germination media tested, burned duff and scarified-shaded duff are somewhat less reliable, and the natural forest floor is the poorest. Seedlings do not grow so rapidly on mineral soil as on the three other kinds of surface material.

(3) Absence of both tree cover and lower plant cover favors establishment and growth during summers in which rainfall is not seriously deficient.

(4) Insects are the chief cause of early mortality. Damage by insects is particularly serious on scarified-shaded duff and undisturbed duff.

In practical terms, it appears that the best conditions for establishment and growth of reproduction of these species will be created by tearing up the ground surface to expose mineral soil and clear-cutting the timber. In order that tree reproduction may be subjected to the least competition from vegetation that invades clear-cut areas, its establishment should be brought about promptly.

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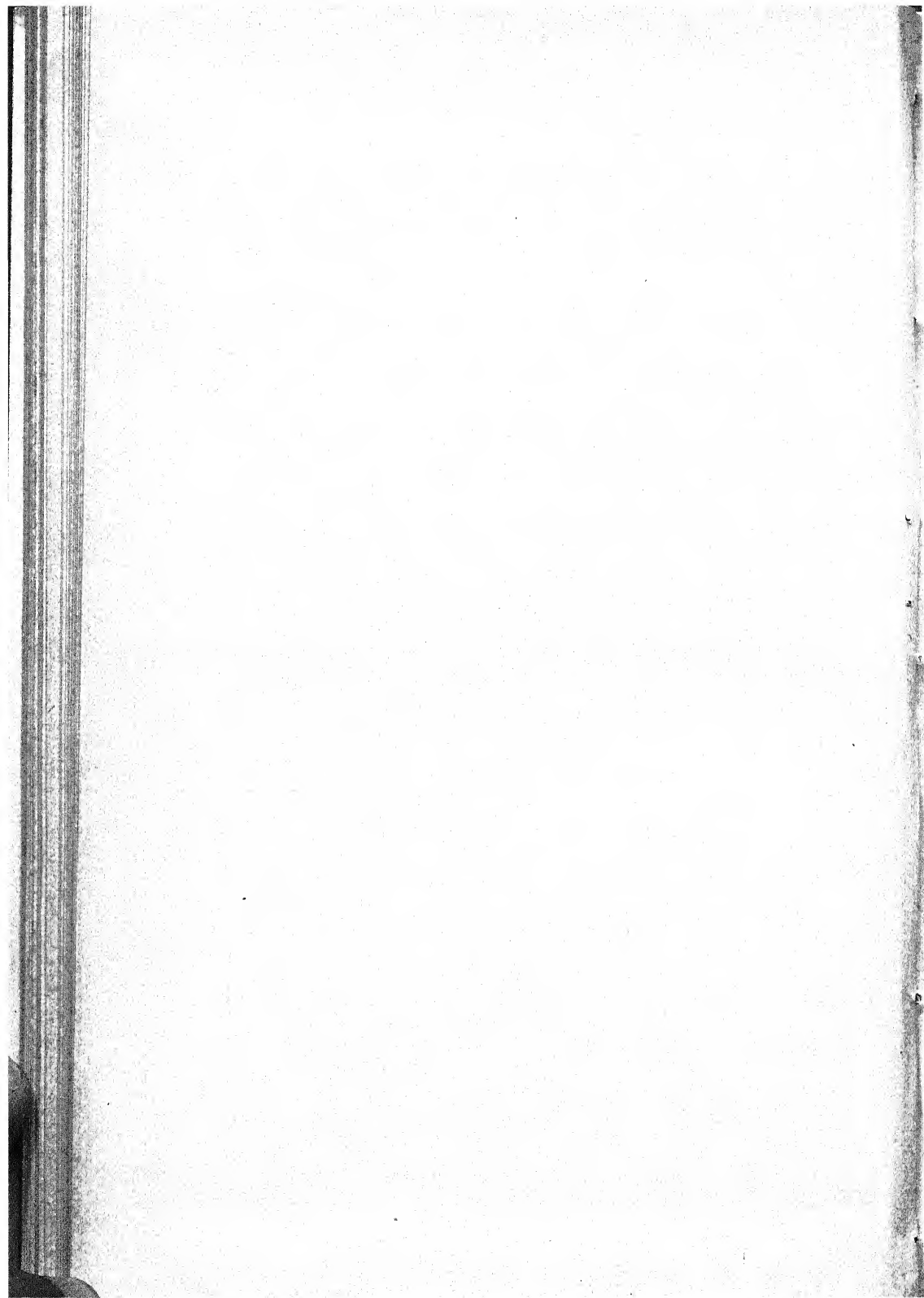
THE EFFECT OF WATER DEFICITS IN THE TREE UPON MATURITY, COMPOSITION, AND STORAGE QUALITY OF BOSC PEARS [1]

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INTRODUCTION

Information concerning the effects of water supply during the growing season on the storage and dessert qualities of tree fruits is rather limited. Such reports as are available were reviewed in a previous publication (1).[2] In adobe clay Anjou pears were, in general, slightly more astringent, more acid, and more highly flavored when appreciable water deficits occurred near harvest than when water deficits were minimized by more frequent irrigation (1). The effects of continued, appreciable water deficits on Bartlett pears in both adobe clay and sandy loam were a slight increase in acidity and flavor and a marked reduction in core break-down (6).

Although Bosc pears are grown principally in the Hood River and Rogue River Valleys in Oregon, and to a lesser extent in Eldorado, Santa Clara, and Placer Counties in California, where summer irrigation is necessary, the relation of irrigation to the high quality that Bosc pears can attain has not been determined previously. In 1936 and 1937 the effect of irrigation upon the quality of Bosc, as well as of Anjou (1) and Bartlett (6), was studied in the Rogue River Valley. The effects of water deficits upon the flavor, firmness, water and sugar content, acidity, and carbon dioxide output of Bosc pears during storage are reported herein.

MATERIAL AND METHODS

ESTABLISHMENT OF PLOTS

Plots of Bosc pears were established in the Kenly orchard, located on the floor of the Rogue River Valley, where the soil is classified as Meyer adobe clay, and in the Topsides orchard, located in the rolling foothills on the west side of the Valley, where the soil is classified as Siskiyou coarse sandy loam. The trees in the Kenly orchard were large and moderately vigorous; those in the Topsides orchard were smaller but moderately vigorous. In each orchard three trees were located on a wet plot and three on a dry plot.

The irrigation needs for the wet plots were determined by measuring 45 tagged fruits (15 per tree) per plot every 3 or 4 days and calculating the rate of enlargement. Water was applied whenever the growth rate of the fruits began to slacken. A midseason irrigation was applied to the dry plot on sandy loam in 1936, but this plot was not irrigated in 1937. The dry plot on adobe clay was not irrigated in either season.

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[1] Received for publication December 1, 1942.
[2] Italic numbers in parentheses refer to Literature Cited, p. 133.

SELECTION OF FRUIT SAMPLES

Pickings for the determination of storage and dessert quality and chemical composition were made at definite intervals after full bloom. During 1936 the pickings were made 135, 145, and 155 days after full bloom, and in addition an attempt was made to harvest fruit from each plot when it reached a firmness of 14, 13, and 12 pounds as measured by the United States Department of Agriculture pressure tester with the $\frac{5}{16}$-inch plunger. Picking according to pressure test did not appear to be satisfactory; so during 1937 pickings for the determination of quality were confined to 5- to 10-day intervals 126 to 155 days from full bloom.

At 10-day intervals, beginning 126 days after full bloom, a packed box from each plot was placed in storage at 31° F. for later ripening and examination. During November part of the fruit from each box was removed to a warm room (65° to 70°) for ripening, in order that quality might be determined during the period when prime dessert quality might be expected. Additional samples were ripened at later dates for comparison of the decline in dessert quality and the development of physiological disorders.

To measure fruit firmness, representative samples of 15 fruits from each plot were selected at 3-day intervals during a period beginning considerably in advance of commercial harvest. Two determinations were made on peeled areas on the sides of each fruit by means of the pressure tester previously mentioned.

For the determination of chemical composition samples of 20 representative fruits were selected at 10-day intervals. Sections for dry weight were obtained by means of a 6-mm. cork borer. Transverse sections removed from the equatorial region of each fruit were trimmed of core and epidermal tissue and dried for 72 hours at 70° C. without vacuum. Samples for chemical determination were removed with a 15-mm. cork borer in the same manner. After removal of core and epidermal tissue the plugs were ground in a food chopper and a weighed amount of the tissue was preserved in alcohol for sugar determination. The juice was recovered from the remaining tissue by straining through several layers of cheesecloth; aliquots of this juice were used for the determination of pH and titratable acidity.

ANALYTICAL METHODS

Titratable acidity was determined on a 25-ml. sample of juice by dilution with 75 ml. of distilled water and by titration with N/10 sodium hydroxide; methyl red was used as the indicator.

Reducing sugars were determined by the Quisumbing and Thomas (5) reduction procedure and the Shaffer and Hartmann (8) titration technique. Sucrose was determined by difference in copper-reducing power before and after acid inversion. Determination of glucose and levulose was accomplished by using the iodine-oxidation method as developed by Lothrop and Holmes (4).

Hydrogen-ion concentration was measured by comparison with a standard cell in a pH meter with a saturated quinhydrone electrode. The index figure represents the ratio of the dissociated to the total acid present, which, according to Du Toit and Reyncke (2), is of value in the determination of harvest maturity.

Carbon dioxide output of the pears in 31° F. storage was measured by a modified "Truog tower" method as described in a previous publication (*1*).   Determinations were made on duplicate samples of 7 to 8 kg. of fruit of typical size from each plot.   Fruits harvested 145 days from bloom were used, as these were considered to be of optimum maturity.   Measurements were made for a 24-hour period at intervals of approximately 2 weeks throughout the storage period. Values given for the carbon dioxide output represent in each case the average of determinations on duplicate lots of fruit.

PRESENTATION OF RESULTS

GROWTH RATE

Water deficits in the dry plots as compared with those in the wet were indicated when the rate of fruit enlargement in the dry plot remained definitely below that in the wet plot.   Figure 1 shows that in 1936 water deficits in the dry plots in both soils began about 110 days (July 25 to 31) after full bloom and continued through the harvest period.   In 1937 the water deficits in the dry plots commenced about 75 to 90 days (July 5 to 15) after full bloom and continued through the harvest period.   The enlargement curves indicate that fruit growth was more adversely affected by water deficits in the sandy loam than in the adobe clay.   In 1937 there was practically no enlargement of fruit from mid-August to mid-September in the dry plot on sandy loam.

PERCENTAGE OF DRY MATTER AND FIRMNESS

Each year at harvest the percentage of dry matter in the fruit grown on each type of soil was considerably greater for the dry than for the wet plot (fig. 2).   The larger percentage of dry matter was accompanied by a greater firmness of flesh except in the fruit grown on adobe clay in 1936.   The differences in percentage of dry matter were then relatively small, the firmness of the fruit from the dry plot decreasing to that of the fruit from the wet plot during the latter part of the harvest period.   A rather consistent relation was found between water deficit as indicated by the figures for fruit enlargement (fig. 1) and percentage of dry weight and firmness (fig. 2).   The greatest difference in rate of fruit enlargement between wet and dry plots was found in the sandy loam in 1937.   As shown in figure 2, the greatest difference in percentage of dry matter and fruit firmness occurred in fruit from the same plots (sandy loam, 1936 and 1937).

QUALITY OF FRUIT AFTER STORAGE

In both seasons the fruit picked 135 days or less after full bloom was not sufficiently mature for best dessert quality, but fruit harvested 145 and 155 days after full bloom ripened with satisfactory quality when withdrawn from cold storage in November.   In general, fruit from the dry plots was of firmer texture when ripe and consequently less juicy and mellow than fruit from the wet plots (table 1).   The fruit from the dry plots was, however, usually sweeter and more highly flavored than that from the wet plots.

Pears grown on the experimental plots in 1936 ripened normally when withdrawn from cold storage on November 15 and December 20. Fruit of the same lots, however, when withdrawn on March 22, 1937, either failed to soften or softened only slightly and did not develop the characteristically smooth, buttery texture of ripe Bosc fruit. As
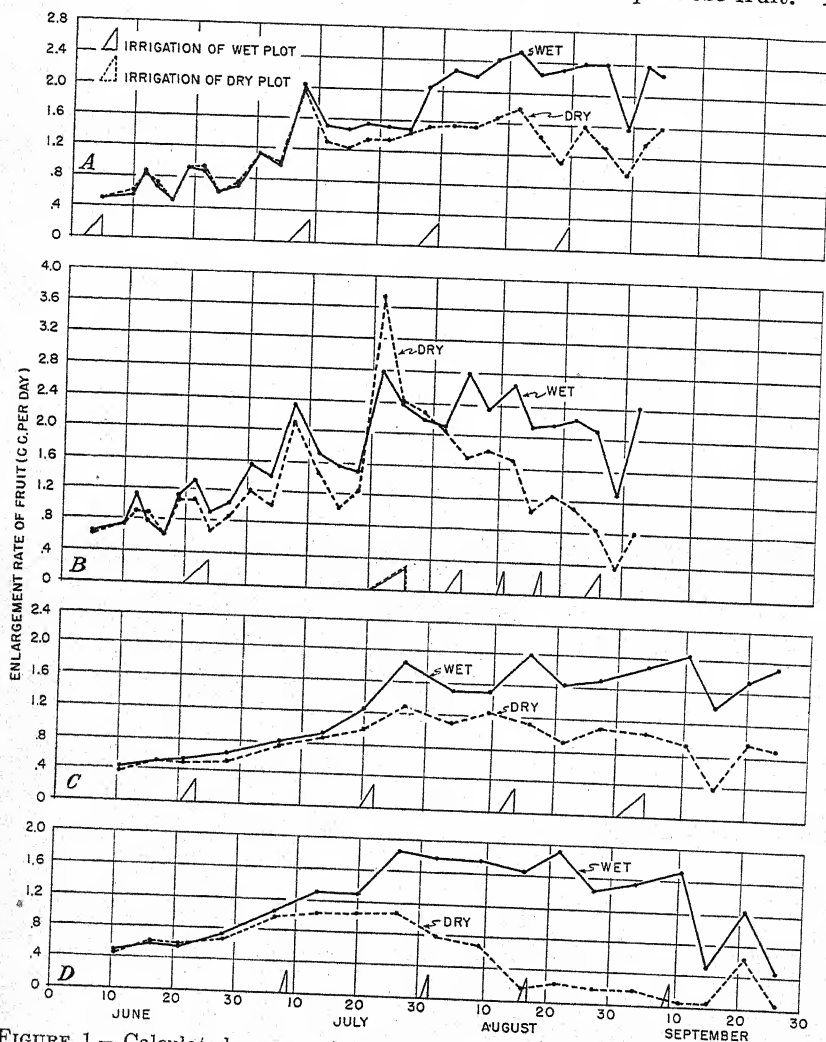
FIGURE 1.—Calculated average daily rate of enlargement of Bosc pears on wet and dry plots: *A*, Adobe clay, 1936; *B*, sandy loam, 1936; *C*, adobe clay, 1937; and *D*, sandy loam, 1937.

shown in table 1, some surface scald appeared on pears from the wet plots in the adobe clay, but none was evident on fruit from the dry plots. This might indicate that senescence was further advanced in the former, but the fact in itself is of little importance, since fruit from both plots failed to ripen normally at this time.
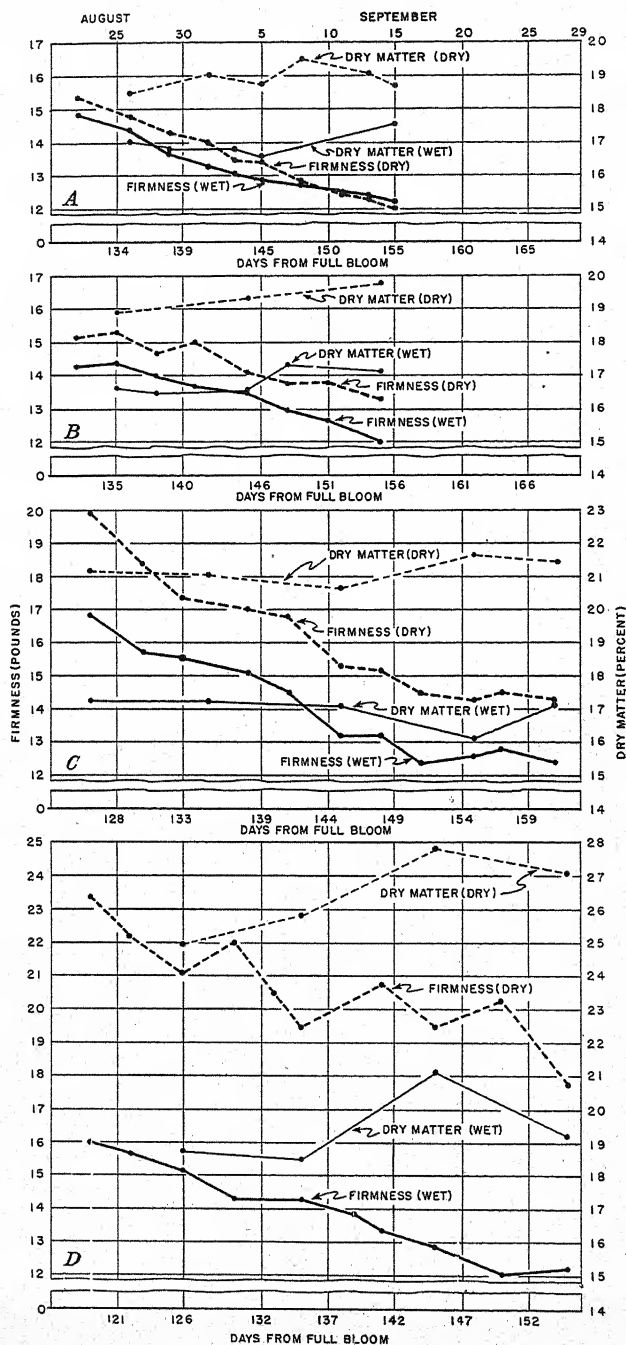
FIGURE 2.—Firmness and dry matter of pears from wet and dry plots: *A*, Adobe clay, 1936; *B*, sandy loam, 1936; *C*, adobe clay, 1937; and *D*, sandy loam, 1937.

TABLE 1.—*Effects of water deficits in the tree upon quality of Bosc pears, 1936 and 1937*

Date of removal from storage and days from bloom to harvest	Kind of plot	Adobe clay				Sandy loam			
		Texture	Flavor	Breakdown	Surface scald	Texture	Flavor	Breakdown	Surface scald
Nov. 15, 1936:[1]									
135	Wet	Fairly juicy, smooth	Sweetness lacking	None	None	Fairly juicy, smooth	Sweetness lacking	None	None.
	Dry	do	Fairly sweet	do	do	Juicy, fairly smooth	Fairly sweet	do	Do.
145	Wet	Juicy, smooth	Sweet, well flavored	do	do	Juicy, fairly smooth	High flavor lacking	do	Do.
	Dry	Fairly juicy, rather firm	Sweet, highly flavored	do	do	do	Sweet, well flavored	do	Do.
155	Wet	Very juicy, buttery	Very good	do	do	Juicy, smooth	High flavor lacking	do	Do.
	Dry	Juicy, rather firm	do	do	do	Juicy, rather firm	Well flavored	do	Do.
Dec. 20, 1936:[1]									
135	Wet	Fairly juicy, smooth	Sweetness lacking	do	do	Fairly juicy, smooth	Sweetness lacking	do	Do.
	Dry	do	Fairly sweet	do	do	Fairly juicy, firm	Fairly sweet	do	Do.
145	Wet	Juicy, smooth	Sweet, well flavored	do	do	Juicy, fairly smooth	Fair	do	Do.
	Dry	Rather granular	do	do	do	Fairly juicy, rather firm	Sweet, well flavored	do	Do.
155	Wet	Juicy, smooth	Very good	do	do	Juicy, smooth	Flavor lacking	do	Do.
	Dry	Rather granular	Highly flavored	do	do	Rather granular	Well flavored	do	Do.
Mar. 22, 1937:[1]									
135	Wet	Crisp, granular		do	Slight	Some softening, but granular.		do	Do.
145	Dry	do		do	None	do		do	Do.
	Wet	do		do	Moderate	do		do	Do.
155	Dry	Slight softening, but granular.		do	Slight	Fairly juicy, granular		do	Do.
Nov. 11, 1937:[2]									
126	Wet	Juicy, fairly smooth	Fairly sweet, lacking flavor.	do	do	Juicy, fairly smooth	Sweetness lacking, astringent.	do	Do.
	Dry	Firm, rather granular	Sweet, fair flavor.	do	do	Granular, firm	Fairly sweet, astringent.	do	Do.
135	Wet	Juicy, smooth	do	do	do	Juicy, smooth	Fairly sweet, lacking flavor.	do	Do.
	Dry	Juicy, firm	Sweet, good	do	do	Granular, dry, very firm.	Sweet, well flavored	do	Do.
145	Wet	Juicy, smooth	Sweet, well flavored	do	do	Juicy, smooth, dry	do	do	Do.
	Dry	Juicy, firm	Very sweet, highly flavored.	do	do	Granular, firm, dry		do	Do.
155	Wet	Fairly juicy, somewhat mealy.	Sweet, well flavored	do	do	Juicy, smooth	Sweet, mild, well flavored.	Slight	Do.
	Dry	Juicy, firm	do	do	do	Granular, firm, dry	Sweet, rather flat.	None	Do.

Dec. 11, 1937:[2]								
126	Wet	Mostly crisp, granular	Fairly sweet, poor	do.	Mostly granular	Poor	do.	Do.
	Dry	Firm, crisp, dry	Fair	do.	Firm, granular, dry	Fair	do.	Do.
135	Wet	Mostly crisp, granular	Sweet, fair	do.	Slightly juicy, crisp	do.	do.	Do.
	Dry	Firm, crisp, dry	do.	do.	Granular, dry	do.	do.	Do.
145	Wet	Mostly granular, crisp	Fair	do.	Some fairly juicy, many granular.	do.	do.	Do.
	Dry	Firm, dry	Sweet, good	do.	Granular, dry	Good	do.	Do.
	Wet	Slightly juicy, many crisp.	Sweet, fair	do.	Some fairly juicy, many granular.	do.	Moder-ate.	Do.
155	Dry	Mostly firm, granular	Sweet, good	do.	Granular, dry	Fair	Slight.	Do.

[1] Placed in cold storage Aug. 26, Sept. 5, and Sept. 15, 1936.
[2] Placed in cold storage Aug. 23, Sept. 1, Sept. 11, and Sept. 21, 1937.

As shown in table 1, fruit produced during the 1937 season, regardless of irrigation treatment, failed to ripen normally when withdrawn from cold storage on December 11. No surface scald and only a small amount of break-down were apparent after ripening, but the fruit was largely granular in texture and much of the desirable flavor had been lost.

Regardless of the period the fruit was held in cold storage all appeared to be in good condition when removed to the ripening room. Ripening response in the warm room was the only reliable index of quality after cold storage. Water deficits during the growing season had no effect on the length of the storage life, as judged by ripening response, that could be detected by the three withdrawals from cold storage in the 1936 experiment or by the two withdrawals in the 1937 experiment.

RESPIRATORY ACTIVITY

As shown in figure 3, carbon dioxide production per unit of dry weight of fruit was appreciably greater for fruit from the dry plot than for that from the wet plot. In general, differences between plots were more pronounced in 1937 than in 1936, probably because water deficits in the dry plot were more severe in 1937 than in 1936. Since the fruits from the dry plots were higher in percentage of dry matter than fruits from comparable wet plots, expressing carbon dioxide production in terms of fresh weight instead of dry weight increased the differences shown in figure 3.

CHEMICAL COMPOSITION OF FRUIT

The glucose, fructose, and sucrose present in the fruit at harvest-time are shown in table 2. The percentage of glucose was in all cases lower at the second than at the first picking, but it did not change appreciably after the second picking. Sucrose continued to increase throughout the harvest period, while fructose showed little change.

Fructose and sucrose were consistently lower, when expressed as percentage of dry weight, in fruits from dry than in fruits from comparable wet plots.

On the other hand, there was no significant difference in the percentage of glucose in fruits from the wet and the dry plots. Thus, the ratio of fructose to glucose was lower for the latter.

The decrease in acidity of fruit with advance in maturity during the harvest period, measured both as titratable acidity and as hydrogen-ion concentration (table 3), was not appreciably influenced by water deficits in the summer of 1936.

As shown in table 3, some effect of water deficits upon titratable acidity is indicated in the 1937 season. Since acidity was determined on expressed juice in 1937 rather than on fresh tissue as in the 1936 season, the higher titratable acidity found in fruits from the dry plots in 1937 is probably significant. Fruits from wet and dry plots on sandy loam showed the greatest difference, and, as previously mentioned, the dry plot on sandy loam suffered the greatest water deficits.

The index figures, as shown in table 3, appear to be of little significance, because in most cases the highest index figure did not coincide with the most desirable maturity. In several cases the highest index figure was obtained for fruits of the first picking in 1937, which were distinctly immature.
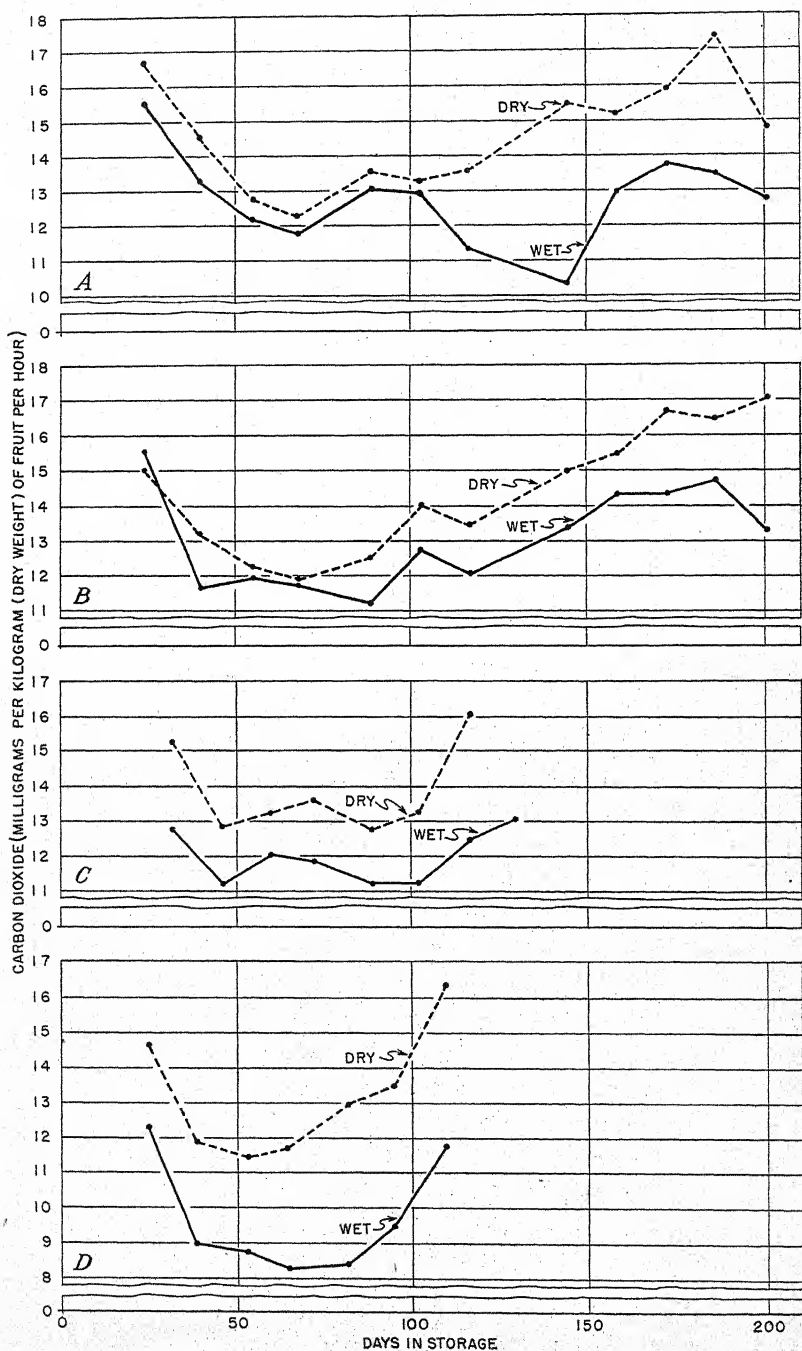
FIGURE 3.—Carbon dioxide output of pears (dry-weight basis) grown on wet and dry plots and stored at 31° F.: *A*, Adobe clay, 1936; *B*, sandy loam, 1936; *C*, adobe clay, 1937; and *D*, sandy loam, 1937.

TABLE 2.—*Effects of water deficits in the tree upon carbohydrate content of Bosc pears, 1936 and 1937*

Kind of soil and date of harvest	Period from bloom to harvest	Wet plot						Dry plot					
		Fresh-weight basis			Dry-weight basis			Fresh-weight basis			Dry-weight basis		
		Glucose	Fructose	Sucrose (as invert)	Glucose	Fructose	Sucrose (as invert)	Glucose	Fructose	Sucrose (as invert)	Glucose	Fructose	Sucrose (as invert)
Adobe clay:													
1936	*Days*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Aug. 26	135	1.60	3.31	2.28	9.39	19.44	13.33	1.55	3.86	1.88	8.37	20.84	10.10
Sept. 5	145	1.10	4.23	2.62	6.63	25.50	15.79	1.25	4.24	2.25	6.67	22.61	11.95
15	155	1.10	4.23	3.48	6.27	24.10	19.83	1.33	3.99	3.41	7.12	21.36	18.23
1937													
Aug. 23	126	1.35	3.41	1.52	7.83	19.80	8.82	1.69	3.38	1.54	8.00	16.00	7.28
Sept. 1	135	1.10	3.55	2.25	6.38	20.60	13.02	1.21	3.66	2.30	5.75	17.38	10.92
11	145	1.05	3.89	3.03	6.15	22.80	17.17	1.07	3.83	3.36	5.17	18.48	16.16
21	155	1.22	3.99	3.06	7.57	24.77	19.03	1.27	3.76	3.50	5.87	13.37	16.18
Sandy loam:													
1936													
Aug. 25	135	1.65	3.57	1.61	9.91	21.44	9.67	1.82	3.69	1.49	9.62	19.51	7.89
Sept. 4	145	1.22	3.96	2.18	7.36	23.88	13.15	1.55	3.81	2.39	8.01	19.69	12.35
14	155	1.36	3.68	2.63	7.94	21.48	15.35	1.69	3.37	2.96	8.53	17.00	14.93
1937													
Aug. 30	126	1.47	3.91	1.84	7.85	20.89	9.77	2.06	3.19	2.29	8.25	12.77	9.12
Sept. 8	135	1.14	3.87	2.42	6.16	20.92	13.08	1.80	3.35	2.92	6.96	12.95	11.28
18	145	1.33	3.85	3.37	6.28	18.19	15.92	1.93	3.47	3.60	6.92	12.44	12.88
28	155	1.16	3.61	3.83	6.03	18.75	19.90	1.82	3.12	4.07	6.69	11.48	14.99

TABLE 3.—*Effect of water deficits in the tree upon titratable acidity and hydrogen-ion concentration of Bosc pears, 1936 and 1937*

Kind of soil and date of harvest	Period from bloom to harvest	Wet plot			Dry plot		
		N/10 NaOH	pH	Index figure	N/10 NaOH	pH	Index figure
Adobe clay:							
1936	*Days*	*Ml.*		*10^-7*	*Ml.*		*10^-7*
Aug. 26	135	[1]7.5	4.16	19.2	[1]7.7	4.27	14.3
Sept. 5	145	[1]7.3	4.30	14.4	[1]6.8	4.32	14.3
15	155	[1]6.1	4.40	13.5	[1]5.9	4.45	12.3
1937							
Aug. 23	126	[2]10.6	4.23	13.9	[2]12.5	4.32	9.6
Sept. 1	135	[2]8.5	4.40	11.7	[2]10.2	4.44	8.9
11	145	[2]9.0	4.38	11.6	[2]9.2	4.49	8.8
21	155	[2]8.5	4.45	10.4	[2]8.7	4.56	7.9
Sandy loam:							
1936							
Aug. 25	135	[1]8.5	4.40	9.7	[1]8.3	4.42	9.3
Sept. 4	145	[1]7.5	4.33	13.0	[1]7.1	4.38	11.8
14	155	[1]6.8	4.38	12.7	[1]7.1	4.42	10.8
1937							
Aug. 30	126	[2]10.7	4.23	13.8	[2]13.0	4.43	7.1
Sept. 8	135	[2]7.7	4.47	11.0	[2]10.0	4.43	9.3
18	145	[2]7.2	4.52	10.5	[2]8.7	4.78	4.8
28	155	[2]7.5	4.45	11.8	[2]8.7	4.74	5.2

[1] To neutralize 25 gm. of fresh tissue.
[2] To neutralize 25 ml. of expressed juice.

DISCUSSION

In view of the relative severity of the water deficits in the trees on the dry as compared with those on the wet plots, particularly in 1937, it is not surprising that the water deficits so markedly affected the percentage of dry matter, firmness, sugar content, and carbon dioxide output of the fruit. The increased percentage of dry matter and the greater firmness of the fruit experiencing severe water deficits may have been largely or entirely the result of the reduced water content of the fruit. It is probable that a reduced supply of carbohydrates in trees suffering appreciable water deficits accounted for the lower fructose, sucrose, and total-sugar content of the fruit from the dry plots as compared with that of the fruit from the wet plots, on the basis of percentage dry weight. These effects of water deficits in the late summer in decreasing the percentage of total sugars per unit of dry weight are in agreement with those reported for Anjou pears (*1*) and are consistent with the findings of Schneider and Childers (*7*), who reported reductions in the apparent photosynthesis in apple leaves with a limitation in soil moisture.

The fact that the carbon dioxide output of fruit from the dry plots was greater than that of fruit from the wet plots was probably due in part to the smaller size of fruit from the dry plots and the resultant greater number of fruits per unit of weight. As fruit growth on the dry plots was almost the same as that on the wet plots during April, May, and June, the period of cell division in the fruits, it is probable that the smaller size of fruits on the dry plots after June was largely the result of smaller cells rather than of fewer cells. Thus it is perhaps reasonable to assume that the fruit from the dry plot had more cells per unit weight and that this was responsible for the greater metabolic activity per unit weight indicated for fruit from the dry plots. The greater surface area of the fruit from the dry plots per unit of weight may also have been a factor in the greater output of carbon dioxide.

The fact that greater respiratory activity per unit of dry weight in the fruit from the dry plots resulted in no measurable shortening of the storage life as compared with that from the wet plots is consistent with the findings of Gerhardt and Ezell (*3*), who reported no direct correlation between the respiratory activity of winter pears and the length of storage life at 32° F.

The effects of water deficits on firmness of the fruit, as measured by the pressure tester, are important inasmuch as this measurement is one of the principal indexes of maturity. Since the fruit from the dry trees was consistently much firmer than fruit from the normally irrigated (wet) trees, it is apparent that the accepted range of firmness for harvest would not be a trustworthy index for fruit from trees with appreciable water deficits. Ripening tests indicated that, in spite of greater firmness, fruit from the dry plots was as mature as fruit from the wet plots at the same number of days from bloom.

The surprising result is that these effects of severe water deficits were not accompanied by a greater influence upon the quality of the fruit when withdrawn from cold storage and ripened. The extremely severe water deficits in the dry trees in sandy loam in 1937 did result in fruit which ripened with granular, firm flesh rather than with the normal, smooth, buttery texture. This was apparently due, however,

to the exceptionally low water content of the fruit rather than to the loss of ripening response. As a result of water deficits this fruit was too small to meet even the minimum commercial requirements; so such lack of quality would not be expected in commercially packed fruit. Apparently, when the water deficits in the tree during the summer are not sufficiently severe to prevent the fruit's reaching commercially acceptable sizes, such water deficits would be expected to be of only minor importance in influencing the storage or dessert quality of Bosc pears.

There was no evidence that Bosc fruits grown on fine-textured soils had better storage qualities than those grown on coarse-textured soils. There was some indication, however, that Bosc fruits grown on the adobe clay were slightly more desirable from the standpoint of flavor and texture than those grown on sandy loam. The wide difference in fruit quality frequently observed in different years was also manifest in the results of these 2 years.

SUMMARY

Bosc pears from trees given moderate and light applications of water were harvested for determination of the effects of water deficit upon the fruit.

Water deficits in the trees on the dry plots were indicated by a consistently reduced rate of fruit growth as compared with that of fruits on the comparable wet plots. Such water deficits began about 75 to 110 days after full bloom and continued through the harvest period.

Percentage of dry matter, determined on the freshly harvested fruits, was higher in fruits from the dry plots than in those from the wet plots. Frequently, when the firmness of fruits from the dry plots was several pounds above the accepted range for picking, the pears were actually mature, as judged by quality when subsequently ripened. Thus, where appreciable water deficits in the trees occur, firmness standards developed for fruit from adequately irrigated trees may not be satisfactory indexes of maturity.

Pickings for storage and quality determinations were made at certain intervals after full bloom, the intervals ranging from 126 to 155 days.

Ripened pears from the wet plots were usually somewhat smoother and mellower in texture, but only in the case of very severe water deficits was the texture of pears from the dry plots unacceptable.

Pears from the dry plots were noticeably sweeter when ripened than those from the wet plots. Pears from the wet plots, however, when ripened early in the season and of proper maturity were sufficiently sweet and well flavored to be readily acceptable to the consumer.

Carbon dioxide output at 31° F., as shown by milligrams of carbon dioxide per kilogram (dry weight) hour, was consistently greater for fruit grown on the dry plots, and the difference was related to the severity of the deficit.

Fructose, sucrose, and total sugars, when calculated on a dry-weight basis, were in general higher in pears grown on wet plots than in those grown on dry plots. Percentage of glucose, calculated on dry weight, was not materially affected by water deficits in the trees.

Titratable acidity and hydrogen-ion concentration were usually somewhat higher in the expressed juice of pears from the dry plots than in that of pears from the wet plots.

There was no indication that water deficits during the growing season affected the storage life of Bosc pears.

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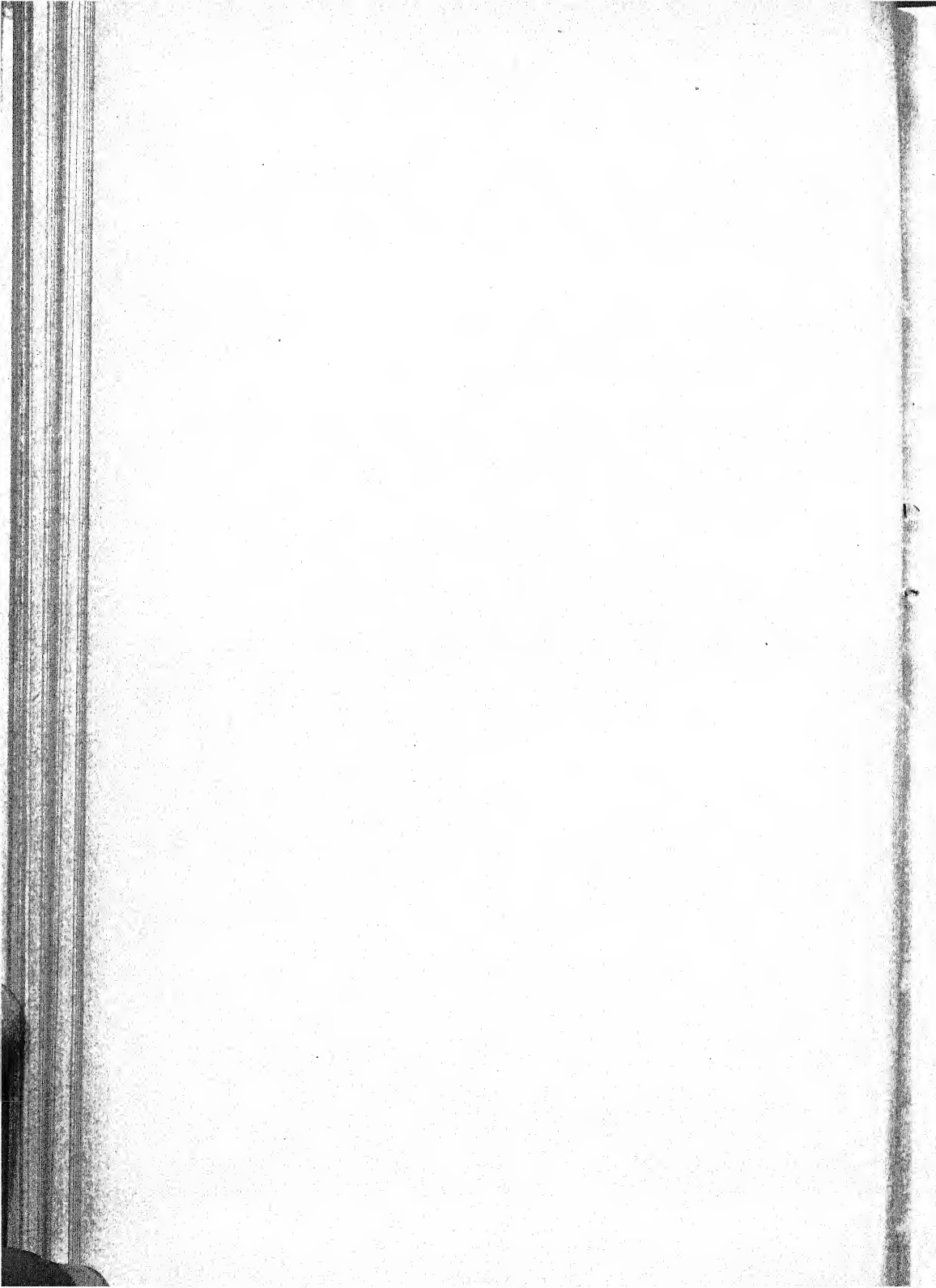
THE AMPHIDIPLOIDS AEGILOPS CYLINDRICA × TRITICUM DURUM AND A. VENTRICOSA × T. DURUM AND THEIR HYBRIDS WITH T. AESTIVUM [1]

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INTRODUCTION

It is generally recognized that the 21 pairs of chromosomes found in the cells of common wheat, *Triticum aestivum* L. (*T. vulgare* Vill.),[2] comprise 3 different sets of 7 pairs each. One of these sets, designated A, is homologous to the 7 pairs of chromosomes that make up the entire complement of *T. monococcum* L. (*n*=7). This is shown by the occurrence of 7 pairs (and 14 unpaired chromosomes) at meiosis in hybrids between *T. aestivum* and *T. monococcum*. Similarly, the 14 chromosome pairs of the emmer wheats (*n*=14), such as *T. dicoccum* Schrank and *T. durum* Desf., are known to be homologous to sets A and B of *T. aestivum*. The third or C set (called D by Japanese workers) does not occur in any wheat with fewer than 21 pairs of chromosomes, but it has been found in *Aegilops cylindrica* Host (*n*=14), a wild relative. Its occurrence in *A. cylindrica* has been detected through observations of chromosome association in hybrids of that species with emmer and *T. aestivum* wheats. In hybrids with the emmers, Bleier (*1*)[3] and others have found usually no pairing between the *Aegilops* and *Triticum* chromosomes; whereas in hybrids with the *T. aestivum* wheats, several investigators, including Sax and Sax (*13*), have usually observed 7 pairs. By producing from *A. cylindrica* and *T. durum* an amphidiploid hybrid containing the full complement of chromosomes from both parents, and then crossing this amphidiploid with *T. aestivum*, it has now been possible to make a more direct test for the presence of the C set of chromosomes in *A. cylindrica*.

Aegilops ventricosa Tausch (*n*=14) resembles *A. cylindrica* in the pairing behavior of the chromosomes in its hybrids with emmer wheats

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(5, 9, 11) and has shown up to seven or more pairs of chromosomes in hybrids with *A. cylindrica* *(4, 11)*. It has given no viable hybrids with *T. aestivum*, but Kihara and Lilienfeld *(7)* have concluded from crosses of it with other species of *Aegilops* having $n = 14$ that it does not possess the C set. This conclusion has now been tested directly, through cytological study of a hybrid between *T. aestivum* and the amphidiploid *A. ventricosa* $\times$ *T. durum*.

MATERIAL AND METHODS

Three varieties of *Triticum durum* were used in making the hybrids from which the amphidiploids were produced. For crossing with *Aegilops ventricosa*, a variety (apparently Mindum) obtained from the Minnesota Agricultural Experiment Station was employed. In the *A. cylindrica* crosses both Acme and F. P. I. No. 94587 [4] were used.

Two forms of *A. cylindrica*, one glabrous and one pubescent, were used in the crosses. Both are referable to *cylindrica* var. *pauciaristata* Eig.

A. ventricosa was all of one type, var. *comosa* (Coss.) Eig.

Two varieties of *T. aestivum*, Chinese and Hope, were used for crossing with the amphidiploids.

For the production of amphidiploids from F_1 material, colchicine treatments as previously described *(14)* were used. Since untreated plants of *Aegilops ventricosa* $\times$ *Triticum durum* set numerous seeds, somatically doubled sectors could not be identified with certainty in this hybrid, and no conclusions could be drawn concerning the efficiency of the various methods of treatment. With *A. cylindrica* $\times$ *T. durum*, the best results were obtained by applying a 0.5-percent aqueous solution of colchicine to cotton-packed crowns of potted plants for a period of 4 days. Treatments of crowns of potted plants with 0.4 percent of colchicine in lanolin for 4 days, or of shoots of very young, unplanted seedlings (with coleoptiles 5 to 10 mm. long) with 0.1 or 0.2 percent of colchicine in lanolin for 1 day, were less successful. A treatment of seedlings with 0.4 percent of colchicine in lanolin was lethal to all 11 individuals to which it was applied.

Cytological observations were made for the most part from fresh acetocarmine smears of material fixed about 2 days in Carnoy's solution. The smears were prepared as described previously *(15)*, except that anthers were frequently fixed singly in order to provide clearer division figures, as recommended by Love *(8)*. Some studies were made from smears made permanent either by the tertiary butyl alcohol technique previously described *(15)* or by the following improved method:[5]

1. Soak off cover slip in 1 part glacial acetic acid to 1 part tertiary butyl alcohol. The cover slip may have to be loosened with a needle if drying has occurred around its edges.
2. Transfer slide and cover slip to pure tertiary butyl alcohol for a few minutes.
3. Add a drop of balsam and replace the cover slip.

The advantages of this method are that immersion oil and sealing material are dissolved by the tertiary butyl alcohol and no destaining occurs in preparations left overnight or longer in either of the fluids.

[4] Accession number of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering.
[5] Developed in collaboration with Dr. Herschel Roman.

HYBRIDS AND AMPHIDIPLOIDS

AEGILOPS CYLINDRICA $\times$ TRITICUM DURUM

The hybrid *Aegilops cylindrica* $\times$ *Triticum durum* and its amphidiploid derivative were intermediate between the two parent species in many characteristics of the spike (fig. 1) and other plant parts.

FIGURE 1.—Mature spikes of (*A*) *Triticum durum*, (*B*, *C*) *Aegilops cylindrica* (glabrous and pubescent, respectively), and (*D–G*) their F_1 hybrids. Glabrous *A. cylindrica* was involved in *D* and *E*, and pubescent in *F* and *G*. Spikes *D* and *F* are 2*n*; *E* and *G*, 4*n*. $\times \frac{3}{4}$.

Little difference was observed between 4n (doubled) and 2n (non-doubled) portions of the same F_1 plant.

TABLE 1.—*Chromosome pairing at first metaphase in F_1 hybrids*

Hybrid	Micro-sporo-cytes examined	Univalents per cell		Bivalents per cell		Cells with univalents only
		Range	Average	Range	Average	
Aegilops cylindrica × *Triticum durum*_____	Number 100	Number 22-28	Number 27.00	Number 0-3	Number 0.50	Number 66
Aegilops ventricosa × *Triticum durum*_____	100	20-28	27.64	0-4	.18	87

Meiotic chromosome pairing in the nondoubled hybrid (table 1) was similar to that reported by other investigators. Most microsporocytes had only univalents at first metaphase, and the bivalents present had only a single, usually terminal chiasma.

Untreated F_1 plants set no seeds. The anthers, which did not dehisce, usually contained over 99 percent of aborted pollen.

The amphidiploid showed a high frequency of univalents at meiosis (table 2), 3.6 per microsporocyte in the most regular plant studied. Multivalent associations were infrequent.

TABLE 2.—*Chromosome pairing at first metaphase in amphidiploids*

Amphidiploid	Micro-sporocytes examined	Univalents per cell		Cells with no uni-valents
		Range	Average	
Aegilops cylindrica × *Triticum durum*_____	Number 50	Number 0-12	Number 3.60	Number 4
Aegilops ventricosa × *Triticum durum*_____	50	0-10	2.56	13

The fertility of the amphidiploid was rather low, the seed set being less than 20 percent, even on the most fertile 56-chromosome plant. The most fertile spike had only 64 percent of seed set, and no other had as much as 50 percent. About 75 to 80 percent of the pollen was normal in appearance.

Chromosome numbers among the offspring of amphidiploid plants were predominantly abnormal, as is shown in table 3. Only 6 out

TABLE 3.—*Chromosome numbers in offspring of amphidiploid plants*

Amphidiploid	Season	Plants grown	Plants examined	Plants with indicated number of chromosomes					
				52	53	54	55	56	57
Aegilops cylindrica × *Triticum durum*_____	1941	Number 18	Number 10	Number 1	Number 2	Number ____	Number 3	Number 3	Number 1
	1942	12	12	____	____	____	7	3	2
Aegilops ventricosa × *Triticum durum*_____	1940	8	5	____	1	1	2	1	____

of the 22 plants classified had 56 chromosomes, and the true frequency of perfectly normal plants may have been even lower. In both the 1941 material, which was grown from seeds on colchicine-treated

plants, and the 1942 material, which came from seeds on two 56-chromosome individuals in the 1941 planting, some of the plants with 56 chromosomes may have had 1 or more monosomes plus an equal number of trisomes. Such abnormalities could have been overlooked in the poorly synapsed metaphases. In the 1941 material, furthermore, only the earliest and most vigorous individuals were examined cytologically, and these were probably the plants that were most nearly normal in chromosome constitution. This possible selection in the 1941 material was offset, however, by the further possibility that some of the seeds used were of nonamphidiploid origin on the female side. Such seeds, produced in $2n$ florets of partly $4n$ spikes, would presumably have tended particularly toward abnormal chromosome constitution, because of their origin through the formation of restitution nuclei rather than through the normal meiotic process.

AEGILOPS VENTRICOSA × TRITICUM DURUM

The hybrid *Aegilops ventricosa × Triticum durum* and its amphidiploid derivative resembled the *A. cylindrica × T. durum* hybrid in being morphologically intermediate between the parent species (fig. 2), in having little $2n$-chromosome pairing (table 1), and in showing a high frequency of univalents in the amphidiploid (table 2). In $2n$ fertility, however, the *ventricosa-durum* hybrid was distinctly higher. Sufficient good pollen (probably between 10 and 25 percent) was present to bring about dehiscence of anthers, and numerous seeds were set from self-pollination. Since the two hybrids were grown in different years, the higher fertility of the $2n$ *ventricosa-durum* material may have been partly an environmental effect, as is suggested by the fact that an F_1 plant of *A. ventricosa × T. durum* grown in the same year as the *cylindrica-durum* material set no seeds. However, this hybrid plant involved a different variety of *T. durum* (F. P. I. No. 94587) from that represented in previous *ventricosa-durum* hybrids. Furthermore, Sorokina (*16*) observed 0.21 percent of seed set in *A. ventricosa × T. durum* hybrids.

In amphidiploid fertility, also, *ventricosa-durum* was higher than *cylindrica-durum*. Several spikes had over 90 percent of seed set, and the entire 56-chromosome plant probably averaged well over 50-percent fertility. Pollen was about 75 to 80 percent normal in appearance.

Of the 5 offspring of colchicine-treated plants examined cytologically (table 3), only 1 had 56 chromosomes. This frequency is similar to that observed in the progeny of *cylindrica-durum* amphidiploids, but there is more reason to suspect that some or all of the *ventricosa-durum* seeds came from nondoubled florets through restituted male as well as female gametes. Although the seeds for planting were selected from the most fertile spikes, there is little assurance that these spikes were entirely $4n$. Some fairly fertile spikes were found also on untreated plants.

Of 7 plants grown from seeds on untreated material, 2 had 55 chromosomes, 3 had 54 chromosomes, 1 had 51 chromosomes, and 1 was not studied. Sorokina (*16*) also obtained some apparently amphidiploid offspring from untreated F_1 plants of *Aegilops ventricosa × Triticum durum*, but did not study them cytologically.
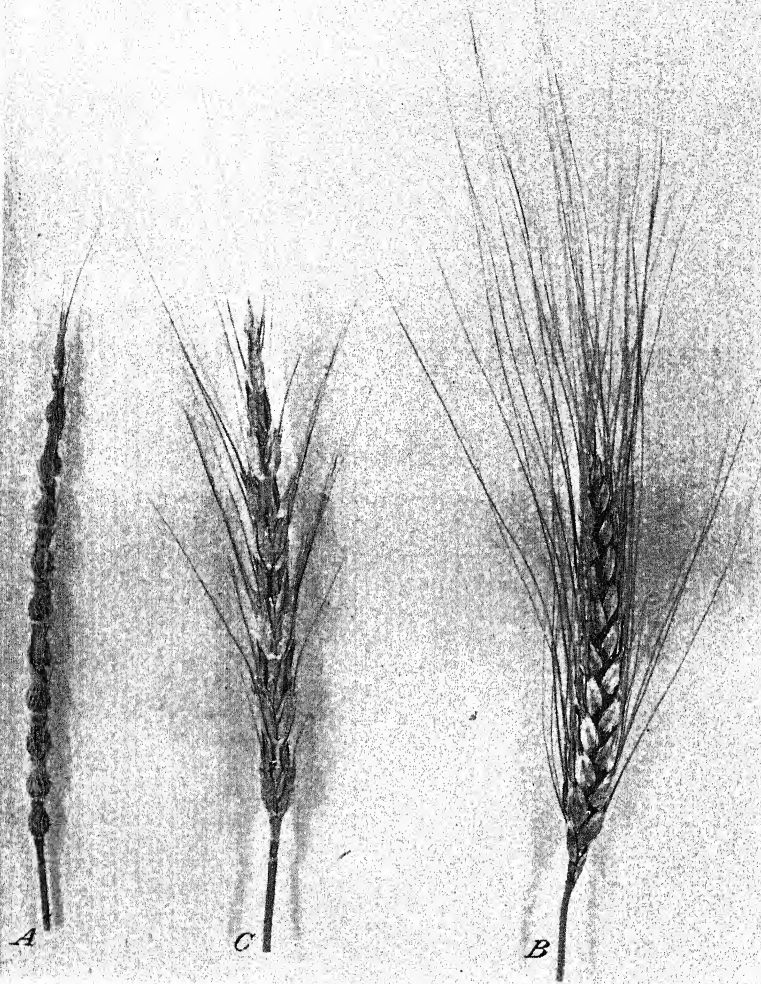
FIGURE 2.—Mature spikes of (*A*) *Aegilops ventricosa*, (*B*) *Triticum durum*, and (*C*) their amphidiploid hybrid. $\times \frac{2}{3}$.

HYBRIDS OF AMPHIDIPLOIDS WITH TRITICUM AESTIVUM

AEGILOPS CYLINDRICA-TRITICUM DURUM × T. AESTIVUM

The cross *Aegilops cylindrica-Triticum durum* × *T. aestivum* was made between a 56-chromosome amphidiploid plant and the variety Chinese of *T. aestivum*. The seed set was comparable to that in the open-pollinated amphidiploid, and of the 8 hybrid seeds tested all were viable. Thirteen seeds of the reciprocal combination were all

inviable. Of the 8 plants grown, 4 were examined cytologically and were found to have the following chromosome numbers: 46, 47, 49, and 49. Only the latter 2 will be considered further.

Studies of chromosome relationships in the hybrids were hampered by the scarcity of analyzable division figures. Some microsporocytes were found, however, which definitely had 21 pairs and 7 univalents (fig. 3). This pairing must represent the association of the A and B sets of *Triticum aestivum* with the chromosomes of *T. durum*, and the association of the C set of *T. aestivum* with 7 of the 14 chromosomes of *A. cylindrica*. The intensity of pairing and the absence of heteromorphic bivalents in cells like the one shown in figure 3 indicated fairly complete homology between the *T. aestivum* C set and 1 *A. cylindrica* set. Trivalents and other multivalents were rare, giving little indication that chromosomes of the second *A. cylindrica* set have much affinity for any of the chromosomes of *T. aestivum*.
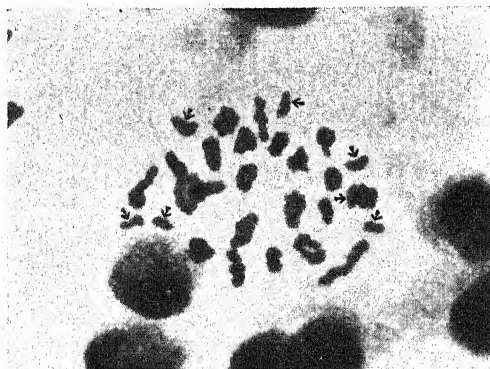
FIGURE 3.—First metaphase in a microsporocyte of (4n *Aegilops cylindrica* × *Triticum durum*) × *T. aestivum*, showing 21 bivalents, 7 univalents. Univalents are indicated by arrows. × 670.

Fertility was low in the amphidiploid-*aestivum* hybrid. Of the two plants available, one had nondehiscent anthers and set no seed and the other had dehiscent anthers containing approximately 25 percent of normal pollen and set four seeds following open pollination (presumably selfing) in the greenhouse. The pollen was functional on both the amphidiploid and *Triticum aestivum*, about one seed per spike being obtained.

TRITICUM AESTIVUM × AEGILOPS VENTRICOSA-T. DURUM

The varieties Chinese and Hope of *Triticum aestivum* were crossed with colchicine-treated F_1 plants of *ventricosa-durum*. Of 9 hybrid seeds involving Chinese, 5 grew; 3 of the resulting plants had 48 chromosomes, and 2 had 47. Of 4 Hope × amphidiploid seeds, 3 grew, and the 2 resulting plants that were examined cytologically had 47 and 49 chromosomes, respectively. All further cytological data were obtained from the 49-chromosome plant.

From 4 to 13 univalents per microsporocyte were observed, averaging 8.7, in 28 cells. If there were no homology between the C set of chromosomes of *Triticum aestivum* and the chromosomes of *Aegilops ventricosa*, 21 univalents would be expected in this hybrid; while com-

plete homology between the C set and 7 chromosomes of *A. ventricosa* would leave 7 chromosomes unpaired. The average occurrence of only 8.7 univalents shows that a considerable amount of homology exists. This homology is imperfect, however, for, besides there being usually more than 7 univalents, frequent trivalents and quadrivalents occurred. No microsporocyte with exactly 21 pairs and 7 univalents was found. It seems likely that only homoeology (i. e., homology involving 1 or more regions of 2 chromosomes but not their entire length) exists between the C chromosomes of *T. aestivum* and the chromosomes of *A. ventricosa*, and that some of the C chromosomes are homoeologous to 2 or more *ventricosa* chromosomes.

Fertility was considerably higher in this hybrid than in *Aegilops cylindrica-Triticum durum* × *T. aestivum*. One open-pollinated (presumably selfed) spike had 9 seeds, a set of about 35 percent. The whole 49-chromosome plant probably averaged close to 10 percent of seed set. Even the plants with 47 and 48 chromosomes set some seeds.

DISCUSSION

The results reported here provide direct confirmation of the assumption that *Aegilops cylindrica* possesses the C chromosome set of *Triticum aestivum*. They also confirm the conclusion of Kihara and Lilienfeld (*7*) that the intact C set is not present in *A. ventricosa*, although the data indicate considerable homoeology between the C set and *ventricosa* chromosomes.

Meiosis in the amphidiploids was characterized by a general reduction in chiasma frequency, usually with the occurrence of univalents. Loss or irregular distribution of these univalents was presumably the chief cause of the low fertility observed. Since these are intergeneric hybrids, their abnormal meiotic behavior might be attributed to incompatibility of the 2 diverse genoms. However, various other amphidiploid hybrids between species of *Triticum* and *Aegilops* have little or no tendency toward asynapsis. Four synaptic amphidiploids with $n=14$ have been reported by the writer (*15*), and 1 more is now available in this group (*A. bicornis* (Forsk.) Jaub. and Spach × *T. monococcum*). Also of good synaptic behavior are amphidiploids of *T. dicoccoides* Körn. ($n=14$) with *A. caudata* L. ($n=7$), *A. sharonensis* Eig ($n=7$), *A. speltoides* Tausch ($n=7$), and *A. umbellulata* Zhuk. ($n=7$), although *T. dicoccoides* × *A. comosa* Sibth. and Smith ($n=7$) had considerable asynapsis.[6] There is reason to doubt, therefore, that incompatibility of diverse genoms is responsible for the poor pairing in the *cylindrica-durum* and *ventricosa-durum* amphidiploids. It seems more probable that the large number of chromosomes hinders pairing in some way. All other 56-chromosome wheat amphidiploids reported have tended to have univalents at meiosis and to be of reduced fertility: *A. ovata* × *T. dicoccoides* (*6, 17*), *A. ovata* × *T. durum* (*17*), *A. ovata* × *T. turgidum* (*12*), and *T. aestivum* × *Secale cereale* (*10*).

Of the hybrids of the two amphidiploids with *Triticum aestivum*, the one involving *ventricosa-durum* was distinctly the more fertile, in spite of its less regular meiosis. It had a frequency of univalents substantially the same as did the hybrid involving *cylindrica-durum*, and had multivalent configurations as well. There is some reason to suspect,

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[6] Unpublished data.

however, that it had more regular pairing of members of the A and B sets of chromosomes with their homologues, since somewhat better pairing occurs in the *ventricosa-durum* amphidiploid than in *cylindrica-durum*. This greater regularity of A and B pairing would result in a higher proportion of *aestivum × ventricosa-durum* gametes with a full complement of A and B chromosomes. Evidence from other hybrids indicates that the possession of complete sets of both A and B chromosomes is ordinarily essential for gametic viability.

Further interest is attached to these amphidiploids because of their possible practical value. Much improvement in *Triticum aestivum*, particularly with respect to disease resistance, has been attained through hybridization with *T. durum* and *T. dicoccum*. No use has ever been made of *Aegilops cylindrica* and its C chromosome set, however, although Johnston (2) and Jones (3) have shown that *A. cylindrica* is highly resistant to leaf rust and hessian fly. If the resistance factors lie in the C set of *cylindrica* chromosomes, it should be possible, by means of relatively simple crossing and selection procedures, to transfer part or all of the resistance of the *cylindrica-durum* amphidiploid to *T. aestivum*.

The *ventricosa-durum* amphidiploid may also be of practical value, since Jones found that *Aegilops ventricosa* also is resistant to hessian fly. Although no perfect homologues of the *Triticum aestivum* C chromosomes exist in *A. ventricosa*, the resistance factors may be transferable to C chromosomes through homoeologous pairing and crossing over.

SUMMARY

By treating hybrids of *Aegilops cylindrica × Triticum durum* and *A. ventricosa × T. durum* with colchicine, amphidiploid sectors were obtained. In addition, *A. ventricosa × T. durum* produced a number of seeds on untreated plants.

Chromosome pairing in the amphidiploids was poor and fertility was low, particularly in *cylindrica-durum*. Aneuploid chromosome numbers predominated among the offspring.

Hybrids of the amphidiploids with *Triticum aestivum* (*T. vulgare*) confirmed the previous assumption that *A. cylindrica*, but not *A. ventricosa*, possesses homologues of the C set of *T. aestivum* chromosomes. Considerable homoeology was indicated, however, between *A. ventricosa* chromosomes and the *T. aestivum* C set.

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EFFECT OF FERTILIZER, SOIL COMPOSITION, AND CERTAIN CLIMATOLOGICAL CONDITIONS ON THE CALCIUM AND PHOSPHORUS CONTENT OF TURNIP GREENS [1]

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INTRODUCTION

Many investigations have been reported which demonstrate that environmental factors are responsible for wide variations in the composition of plants. These investigations, however, furnish little information concerning the effects of specific factors on the nutritive value of vegetables. Field studies relating to the production of vegetables have been chiefly concerned with yield and marketability of the product rather than with its nutritive value. Greenhouse studies have been conducted to determine the effects of certain environmental factors on the composition of vegetables, but the extent to which the results of such studies can be applied to field crops is questionable. Reported studies thus do not provide adequate information which might serve as a basis for recommending cultural practices designed to increase the nutritive value of food plants.

The experiments reported here were undertaken as one phase of a cooperative project to determine some of the most important factors which affect the nutritive value of vegetables. It was believed that a study in which the same varieties of vegetables were grown in different geographical areas where soil and climatic conditions varied widely would furnish information of much more value than could be obtained if the vegetables were grown in only one area. The purpose of the present study was to determine the effect of fertilizer treatments, soil composition, and certain climatological conditions on the calcium, phosphorus, and iron content of turnip (*Brassica rapa* L.) greens. The results on the calcium and phosphorus content of turnip greens are reported in this paper.

MATERIAL AND METHODS

PLAN OF EXPERIMENTS

Two different plans have been followed in the experiments on turnip greens. In the first series of experiments the fertilizer treatment was uniform in each locality, except at two places in Texas where fertilized and unfertilized treatments were compared, and replications alone were used. This plan was changed to one of factorial design, because of the greater efficiency of the latter. The experiments will be referred to as nonfactorial and factorial experiments.

The nonfactorial experiments were conducted during the fall of 1938 in Mississippi, South Carolina, Texas, and Virginia, and continued in South Carolina in the spring of 1939. A total of 19 experiments in 12 localities were carried out. The soil was fertilized in the manner customary for the locality; the sources for nitrogen, phosphorus, and potassium were nitrate of soda, superphosphate, and muriate of potash, respectively. No other fertilizers were used. Five crops at 3 places in Texas received no fertilizer treatment. Applications of fertilizer on the acre basis at the 11 places were as follows: In Mississippi, 1,000 pounds of 6–8–4 were used at Poplarville, Natchez, and West Point, and 600 pounds of nitrate of soda at Stoneville; in Norfolk, Va., 1,000 pounds of 6–6–5; in South Carolina, 500 pounds of 4–8–4 at Edisto, 800 pounds of 4–12–4 at Clemson, and 1,000 pounds of 4–8–4 at Sandhill; in Texas, 150 pounds of 11–48–0 and no fertilizer at Winter Haven, 200 pounds of 6–12–6 and no fertilizer at College Station, and no fertilizer at Iowa Park, or at Lubbock. The plantings consisted of 20 rows, 20 feet long, of which the inner 10 rows 10 feet long were harvested for sampling. Each row constituted a separate sample.

The factorial experiments were conducted during the spring and fall of 1939 and 1940. A total of 30 experiments in 19 localities situated in Georgia, Mississippi, Oklahoma, South Carolina, Texas, and Virginia, were carried out. A $2 \times 2 \times 2 \times 2$ factorial design was used consisting of 16 treatments with 2 replications in each experiment. The 4 fertilizer factors, nitrogen, phosphorus, potassium, and calcium, were studied in all combinations of high and low levels. The check or natural untreated soil constituted the low level for each of the 4 nutrients. The treatments were as follows: NPKCa, NPK, NPCa, NP, NKCa, NK, NCa, N, PKCa, PK, PCa, P, KCa, K, Ca, and check. The acre rates and materials used were: 60 pounds of N from ammonium sulfate, 60 pounds of P_2O_5 from triple superphosphate, 60 pounds of K_2O from muriate of potash, 120 pounds of Ca from gypsum.

The standard procedure for the factorial experiments was to grow single-row plots 25 feet long and not less than 30 inches apart.[3] The 16 treatments were randomized in a single block with at least 1 guard

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[3] Since the fertilizer was applied in single-row plots, the possibility of cross-feeding between adjacent rows was considered. Weaver and Bruner (27)[4] report the maximum length of any lateral root attained by turnips in Kansas after 111 days through midsummer was 30 inches. This growth period was equalled in only one instance (at Blacksburg, Va., 110 days) under much more favorable moisture conditions. Under similar conditions of moisture at Geneva, N. Y., Goff (13) observed horizontal roots no longer than 18 inches. Moisture conditions, growth period, and distance between treatments were thus unfavorable for cross-feeding. In addition, the clean cultivation given the plots discouraged the development of long lateral roots. General observation of the wide difference in growth response of various annual crops on adjacent fertilized and unfertilized rows greatly strengthens the assumption that there can be little cross-feeding under such circumstances. This was noted especially for those plots lacking nitrogen at many locations east of the Mississippi River.

[4] Italic numbers in parentheses refer to Literature Cited p. 189.

row on each side. A duplicate block with a different arrangement of treatments was grown, usually adjacent to the first, but in some cases it was necessary to separate the 2 blocks to secure uniformity of growing conditions. The fertilizer was applied in a band 2 inches to the side and 1 inch below the seed level.

GROWING THE CROP

The Seven Top variety of turnip was chosen because it represents a type that is grown primarily for greens rather than for the root. It is well adapted and makes a good growth under favorable conditions over the entire region included in this study. A uniform lot of seed was supplied by the United States Regional Vegetable Breeding Laboratory at Charleston, S. C.

The extent of the region covered by the cooperating States provided opportunity to grow greens under a wide variety of soil types, rainfall, and temperature. Soils typical of each area were selected for the experiment. Each site was chosen as being representative of home-garden conditions at each location. When crops were grown for successive seasons in the same locality, planting was not made twice on the same site, but on one nearby having the same kind of soil. The places where greens were grown and the average annual precipitation in inches for each site are shown in figure 1.[5] The cultural history for the locations is summarized in table 1. The soil types, given for each location in table 2, ranged from the comparatively deep Norfolk fine sand of the Sandhill section of South Carolina to the Houston clay-loam of northeastern Mississippi. The following great soil groups are represented: Gray-Brown Podzolic soils, Red and Yellow Podzolic soils, Reddish Chestnut soils, Reddish-Brown soils, Rendzina soils, and Ground-Water Podzolic soils. Records of the rainfall and temperature during the growing season at each locality are shown in table 3.
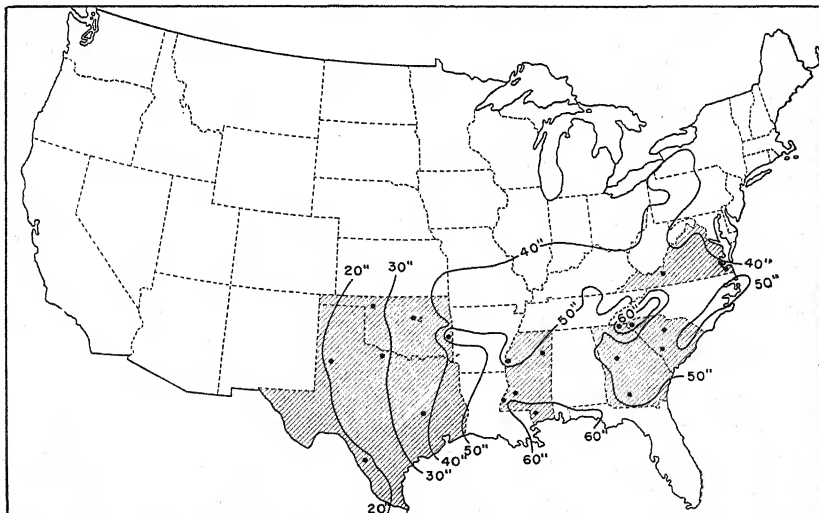
FIGURE 1.—Average annual precipitation in inches for the 20 places in which turnip greens were grown.

[5] See KINCER, J. B. PRECIPITATION AND HUMIDITY, *In* Baker, O. E., Atlas of American Agriculture, pp. 6, 7. Illus. 1922.

TABLE 1.—*Location and recent cultural history of sites where turnip greens were grown*

State	Location	Year	Crop or cultural treatment	Commercial fertilizer [1]	Barnyard manure (Tons)	Green manure
Georgia	Blairsville	1929	Lettuce	800 pounds 8-8-6		Winter rye and vetch.
		1940	Potatoes	do		Austrian winter peas.
	Experiment	1938	Tomatoes	600 pounds 8-8-6	10-20	Winter rye and vetch.
	Tifton	1939	Pimientos	300 pounds 4-8-10	10-20	Do.
	Crystal Springs	1939	Peanuts	Complete fertilizer		Winter cover of crotalaria.
		1939	Miscellaneous vegetable crops	do		
		1940	...do			
Mississippi	Natchez	1936	Tomatoes	1,000 pounds 4-8-4		
		1937	Watermelons	do		
	Poplarville	1938	Cabbage	do	8-10	
		1933-38	Winter oats (followed by cowpeas or soybeans for hay).			
	Stoneville	1935-36	Soybeans			
		1937-38	Tomatoes (followed by nonfactorial experiments).		12-15	
		1938-39	Sweetpotatoes			
	West Point	1939	Spring crop of potatoes (followed by factorial experiment).	100 pounds ammonium sulfate.		
		1939	Summer eggplant (followed by factorial experiments).	do		
		1934-37	Alfalfa	300 pounds 18 percent superphosphate.		
Oklahoma	Heavener	1938	Corn	Phosphate		
	Perkins	1939	Corn			
		1910	Mung beans			
	Woodward	1939	Fallow			
		1940	Dwarf milo, cowpeas			
South Carolina	Clemson	1936-37	Wheat			
		1938	Asparagus seedlings			
	Edisto	1938	Fallow	1,000 pounds 5-10-5		
		1939	Cantaloupes	400 pounds 5-7-5		
	Sandhill	1938	Okra	500-700 pounds 4-8-4		
		1938	Watermelons	do		
		1939	Rye (followed by nonfactorial experiment).			
		1939-40	Fallow (followed by factorial experiment).			
	College Station	1938	Tomatoes	300 pounds 6-12-6		Winter oats.
		1940	Squash	do		
	Iowa Park	1938	Garden			
Texas	Lubbock	1933-35	Trees and shrubs (nursery).			
		1938	General vegetable crop.			
	Winter Haven	1938	English peas			
		1939	Spinach			
		1940	Sugar beets			
Virginia	Blacksburg	1935-40	Grass	No residual effect.		
		1939	Strawberries	Residual effect of N.		
	Norfolk	1940	Spinach			

[1] No lime had been applied to any site with the exception of that at Sandhill where there had been applications of 1,000 to 1,500 pounds per acre.

TABLE 2.—*Data on composition of soil samples taken from turnip-green plots of the factorial experiments*

Experiment No.	State	Location	Year	Soil type	pH value	Calcium[1]	Phosphorus[1]	Magnesium[1]	Organic matter (Percent)	Total exchange capacity[2] (Milliequivalents)	Exchangeable calcium[2] (Milliequivalents)
1	Georgia	Blairsville	1939	Conaree sandy loam	5.5	M−	T+	T+	3.43	13.9	3.4
2	do	do	1940	do	5.6	H	L−	T	4.00	17.4	4.1
3	do	Experiment	1939	Cecil sandy clay loam	5.3	T+	L+	L−	3.21	6.7	1.9
4	do	do	1940	do	5.8	T	L+	L−	1.92	6.3	3.8
5	do	Tifton	1939	Norfolk sandy loam	5.6	L	M	L−	1.93	3.2	1.7
6	Mississippi	Crystal Springs	1939	Grenada silt loam	5.4	L	L−	M	1.22	5.4	1.1
7	do	do	1940	do	4.8	L		M	1.33	9.0	1.7
8	do	Natchez	1939[3]	Memphis silt loam	5.4	T	L+	L−	1.07	3.8	.9
9	do	Poplarville	1939	Ruston fine sandy loam	5.4	T+	L	M−	.68	5.0	1.9
10	do	do	1940	do	5.1	T	L−	L−	1.07	4.0	.8
11	do	do	1939	do	7.0	H	H	H	1.36	10.9	3.5
12	do	Stoneville	1939	Sarpy loam	6.8	H+	H	H+	1.55	34.3	4.9
13	do	do	1939	do	7.8	M−	L−	M	1.76	3.8	30.0
14	Oklahoma	West Point	1939	Houston clay loam	6.1	L	T+	M+	1.00	6.2	2.8
15	do	Heavener	1939	Hanceville fine sandy loam	5.1	M+	L−	H	1.30	5.5	2.7
16	do	Perkins	1939	Canadian loamy fine sand	6.0	H	L+	H	1.35	5.0	2.8
17	do	do	1940	do	6.6	H	L+	H	1.20	13.1	4.6
18	do	Woodward	1939	Pratt fine sandy loam	7.4	H−	L+	M−	1.78	10.1	10.8
19	do	do	1940	Weymouth very fine sandy loam	7.6	L−	L+	L	1.40	4.7	8.0
20	do	do[4]	1940	do	6.3	T	L+	H	1.01	3.5	1.6
21	South Carolina	Clemson	1939	Lloyd clay loam	5.7	M	L+	H	1.42	2.0	1.7
22	do	Edisto	1939	Marlboro sandy loam	5.5	H	L+	M−	1.00	5.5	.9
23	do	Sandhill	1940	Norfolk fine sand	5.4	H	M−	L	.48	7.9	2.9
24	do	College Station	1940	Lufkin fine sandy loam	8.6	T+	M−	H	.84	12.2	11.8
25	Texas	Iowa Park	1939	Yahola loamy very fine sand	7.2	L−	M+	H	1.29	7.8	5.4
26	do	Winter Haven	1939	Orelia clay loam	7.7	H	L	H	.84	7.2	1.6
27	do	do	1939	do	7.7		L+	L−	2.42		2.3
28	Virginia	Blacksburg	1940	Dunmore silt loam	5.5	L−	L+	L	2.47	7.2	3.7
29	do	Norfolk	1939	Keyport sandy loam	5.8	H	L+	M	2.65	7.1	
30	do	do	1940	do	6.9						

[1] T (trace)=0–300 pounds Ca, 0–10 pounds P, and 0–10 pounds Mg per acre; L (low)=300–1,000 pounds Ca, 10–50 pounds P, and 10–30 pounds Mg per acre; M (medium)=1,000–2,000 pounds Ca, 50–100 pounds P, and 30–90 pounds Mg per acre; H (high)=2,000–3,000 pounds Ca, 100–250 pounds P, and 90–150 pounds Mg per acre; H+ (very high)=3,000–4,000 pounds Ca, 250–400 pounds P, and 150–200 pounds Mg per acre.

[2] Milliequivalents per 100 gm. of soil.

[3] Spring crop.

[4] Irrigated plots.

TABLE 3.—*Mean temperatures and rainfall for the growing periods of the turnip-green crops for the years 1938, 1939, and 1940*

Average temperature values are in °F.; rainfall in inches.

Location	Growing period (1938)	Max. (1938)	Min. (1938)	Rainfall (1938)	Growing period (1939)	Max. (1939)	Min. (1939)	Rainfall (1939)	Growing period (1940)	Max. (1940)	Min. (1940)	Rainfall (1940)
Georgia:												
Blairsville					Mar. 6–June 8	74.8	48.6	10.32	Apr. 26–June 8	80.5	50.5	5.46
Experiment					Mar. 28–June 16	78.8	57.2	11.11	Aug. 27–Oct. 7	85.2	61.8	4.67
Tifton					Feb. 20–May 8	75.1	53.3	17.94				
Mississippi:												
Crystal Springs	Sept. 17–Nov. 28	82.2	54.5	6.17	Sept. 15–Nov. 20	77.2	53.4	5.67	Mar. 11–May 10	72.3	49.4	14.28
Natchez					Mar. 22–May 16	79.1	53.4	9.88	Feb. 21–Apr. 26	73.2	50.2	11.38
Poplarville	Sept. 6–Nov. 25	82.4	58.2	7.85	Feb. 7–May 8	82.6	59.8	4.42				
Stoneville	Sept. 10–Nov. 25	82.3	53.3	2.83	Oct. 2–Nov. 14	82.6	49.1	4.12	Feb. 27–Apr. 22	70.3	46.4	9.46
West Point	Aug. 29–Nov. 15	82.7	54.1	3.44	Mar. 16–May 18	75.0	50.1	8.76				
Oklahoma:												
Heavener					Mar. 18–May 14	75.2	40.4	11.05	May 6–June 20	83.7	59.6	5.41
Perkins					Apr. 17–May 26	80.0	51.4	5.59	Apr. 26–June 8	82.3	56.6	2.59
Woodward					Mar. 31–May 28	78.7	51.5	5.87	Apr. 26–June 13	82.2	56.6	[1]3.23
South Carolina:												
Clemson	Aug. 15–Oct. 24	83.0	59.2	4.30	Apr. 17–June 24; Aug. 14–Oct. 30	81.1; 84.8	59.7; 58.0	8.32; 8.35				
Edisto	Sept. 5–Oct. 21	82.4	60.4	5.20	Mar. 18–Apr. 25; Sept. 12–Oct. 25	74.6; 84.9	51.3; 62.3	2.21; 2.97				
Sandhill	Sept. 10–Nov. 15	77.6	56.6	3.83	Mar. 15–May 3	73.4	51.7	2.82	Apr. 1–May 27	77.3	53.2	3.70
Texas:												
College Station	Sept. 16–Nov. 11	86.4	55.5	3.54	Aug. 25–Nov. 13	82.5	57.7	4.88	Sept. 14–Nov. 7	84.2	53.4	5.48
Iowa Park	Sept. 5–Nov. 8	87.6	55.6	[1]3.91					Mar. 14–June 6	85.7	33.0	19.74
Lubbock									Oct. 4–Nov. 19	80.4	55.7	[1]2.00
Winter Haven	Oct. 5–Dec. 1	82.1	53.1	[1].08	Nov. 10–Jan. 18	68.5	44.2	[1]2.94				
Virginia:												
Blacksburg									May 8–June 10	65.3	42.6	10.20
Norfolk	Sept. 12–Dec. 12	70.4	50.4	13.64	Aug. 18–Oct. 30	81.9	62.1	9.56	Aug. 14–Sept. 29	81.2	63.6	10.77

[1] Plots received irrigation in addition to recorded rainfall.

The greens were given the cultivation common to good garden procedure, which did not vary much among the different locations. This involved adequate preparation of the seedbed with turning plow, disk, and harrow, and subsequent cultivation and hoeing to keep down weeds. Seeding was comparatively heavy to insure a good stand. It was sometimes necessary to water the fall crop early in the season to get it started. All crops were grown under irrigation at Winter Haven and at Iowa Park, Tex., and one crop at Woodward, Okla.

The use of insecticides was avoided as far as possible and dusts or sprays containing copper or calcium were not used. In cases where aphids or other leaf insects made their appearance late in the season, no control measure was employed. No insects were observed on the plantings in Mississippi and Oklahoma. It was necessary to use rotenone dust for flea beetles at Winter Haven in 1938 and at Iowa Park the following year, early in the growing season. Aphids were found later in the season at other places, but were not serious except at Experiment, Ga., where they appeared earlier, and were controlled by syringing with a garden hose and the use of a soft brush.

SOIL ANALYSIS

Samples of soil for analysis were taken from the sites before treatment. The determinations carried out on each sample and the methods used were as follows: The pH value was estimated electrometrically, using a glass electrode; organic matter by the Schollenberger method or modifications of it (*24, 1, 22*); exchange capacity by the Schollenberger and Dreibelbis methods (*25*); and exchangeable calcium by the Association of Official Agricultural Chemists method (*5*). Calcium, phosphorus, and magnesium were determined by rapid soil tests as used in the North Carolina Soils Testing Laboratory (*15*).[6]

PLANT ANALYSIS

COLLECTION AND PREPARATION OF SAMPLES

The turnip greens were harvested when they were at a good marketable stage, but the length of the growing period varied in different localities (table 3). In the majority of instances all of one crop was harvested in 1 day. The greens were handled as soon as possible after harvesting. A random or a representative sample from each of the 32 plots (16 treatments replicated) was selected for analysis, and damaged leaves or those which would be unfit for cooking were discarded. The petioles of the leaves were cut or broken off about three-fourths of an inch above the crown of the root, and the greens were then sorted and thoroughly washed 3 to 6 times in tap water, or until they were free from soil, and finally washed in distilled water. The excess moisture was drained off, and in some instances there was a preliminary drying in a clean dust-free room before the samples were placed in the oven. A Freas electric oven with a forced draft was used in 4 of the 6 laboratories, and the samples were dried at about 100° C. They were then ground in a ball mill equipped with porcelain jars and flint pebbles, and stored in Mason jars or glass bottles with plastic screw tops.

Duplicate fresh moisture samples were taken from each of the 32 lots of turnip greens in every crop, at the time the samples were being

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[6] MILES, I. E. BUFFERED PERCHLORIC ACID METHOD OF SOIL TESTING. [Unpublished data.]

prepared for analysis. Either randomized plants or leaves, or selected plants, were used. The samples were handled and washed in the same manner as the samples used for analysis. After washing they were spread out and allowed to dry until all visible moisture had disappeared, but they were not allowed to wilt. They were weighed, dried at a temperature of 100° C. or less, placed in aluminum moisture dishes with covers, and dried to constant weight ±0.2 gm. at 100°. A "trip" balance was used in most instances.

For moisture determinations on the stored samples, duplicate 2-gm. samples were weighed into small aluminum moisture dishes with covers. The samples were dried for 5 hours at 100° to 102° C., cooled in a desiccator, and weighed.

METHODS OF ANALYSIS

Methods of the Association of Official Agricultural Chemists were used for both calcium (*3, p. 104*) and phosphorus (*4, p. 21*) determinations, with minor variations in the procedures among the different analysts. The samples were ashed according to the A. O. A. C. method (*4, p. 121*).

Since each State was responsible for the analysis of its own samples, the calcium and phosphorus analyses were made by a number of chemists. For this reason, it seemed advisable to carry out referee work among the different analysts. Accordingly, six composite samples of dried turnip greens were analyzed in duplicate by seven chemists. The statistical analyses of referee data showed conclusively that the value of interaction, laboratory × sample, for both calcium and phosphorus was too small to vitiate the results of the factorial experiments.

NONFACTORIAL EXPERIMENTS

The average calcium and phosphorus content of 10 plots (rows) of turnip greens from each of the 19 nonfactorial experiments, 17 of which were compared on the basis of standard error of the mean (*19*), is shown in table 4. It is evident from an examination of the data that there were marked differences in both the calcium and phosphorus content of the greens grown at different places. The range for calcium was from 1.814 to 4.318 percent and that for phosphorus from 0.3456 to 0.6797 percent. The variations in calcium were greater than those for phosphorus. Thus there were 5 experiments, in which the calcium content was significantly different from that of all other places. For phosphorus this was true only of 1 experiment.

While many factors were probably responsible for variations in the calcium and phosphorus content of the greens, the data indicate that differences within a location occurred as a result of season, fertilizer treatment, and size of the plant. The effect of season is shown by the results of the experiments at Edisto, Clemson, and Sandhill. There were significant seasonal differences when the greens were grown at the same place. In two of the three places, the spring greens contained more calcium and more phosphorus than the fall greens. At Edisto, however, the fall greens had a higher calcium content, and at Sandhill, a higher phosphorus content than the spring greens. There was a significant difference in both the calcium and phosphorus content between greens grown on fertilized and those grown on unfertilized

TABLE 4.—*Calcium and phosphorous content of turnip greens from 19 nonfactorial experiments, 17 [1] of which were compared on the basis of standard error of the mean*

Experiment No.	State	Location	Season	Calcium				Phosphorus			
				Average content, 10 plots (dry basis)	Standard deviation	Standard error of mean	Significantly different from all experiments except Nos.—	Average content, 10 plots (dry basis)	Standard deviation	Standard error of mean	Significantly different from all experiments except Nos.—
				Percent				*Percent*			
1	Mississippi	Natchez	Fall, 1938	2.863	0.118	0.037	7, 14, 17	0.6080	0.0227	0.0072	3, 6, 7, 12, 17, 18.
2	do	Poplarville	do	2.433	.081	.026	(2)	.5696	.0373	.0118	7, 8, 10, 11, 12, 17, 19.
3	do	Stoneville	do	2.206	.120	.038	19	.6300	.0346	.0109	1, 12, 13, 18.
4	do	West Point	do	4.318	.231	.073	(2)	.4360	.0297	.0094	5.
5	South Carolina	Clemson	do	2.655	.041	.013	8, 9, 18	.4430	.0223	.0071	4.
6	do	do [3]	Spring, 1939	3.321	.091	.029	12	.5958	.0261	.0083	1, 2, 7, 12, 17, 18.
7	do	do [4]	do	2.886	.051	.016	1, 14	.5987	.0123	.0039	1, 6, 12, 17, 18.
8	do	Edisto	Fall, 1938	2.696	.046	.015	5, 17	.5641	.0140	.0044	2, 10, 11, 12.
9	do	do	Spring, 1939	2.624	.053	.017	5, 18	.5797	.0044	.0014	13.
10	do	Sandhill	Fall, 1938	1.814	.040	.013	(2)	.5653	.0146	.0046	2, 8, 12.
11	do	do	Spring, 1939	1.900	.026	.008	(2)	.5490	.0183	.0058	2, 8, 19.
12	Texas	College Station [5]	Fall, 1938	3.317	.201	.063	6	.6004	.0544	.0172	1, 2, 3, 6, 7, 8, 10, 17, 18.
13	do	do	do	3.039	.144	.046	(2)	.6688	.0657	.0208	8, 9, 18.
14	do	Iowa Park [5]	do	[6] 2.850	.216	.048	1, 7, 17	[6] .3456	.0536	.0120	(2).
15	do	Lubbock [5]	Fall, 1939	[1] 2.920				[1] .4510			
16	do	do [5]	Spring, 1940	[1] 3.241				[1] .4832			
17	do	Winter Haven [5]	Fall, 1938	2.753	.126	.040	1, 8, 14	.5894	.0274	.0087	1, 2, 6, 7, 12.
18	do	do	do	2.617	.104	.033	5, 9	.6351	.0622	.0197	1, 3, 6, 7, 12, 13.
19	Virginia	Norfolk	do	2.100	.159	.050	3	.5418	.0186	.0059	2, 11.

[1] Lubbock submitted 1 composite sample from 10 plots for each year and is, therefore, not included in the statistical analyses.
[2] No exceptions.
[3] Only plants with small leaves were analyzed; large and small plants from Clemson were from same plot.
[4] Only plants with large leaves were analyzed.
[5] Nonfertilized plots.
[6] Average of 20 plots.

plots at Winter Haven and College Station. The greens from the fertilized plots contained less calcium but more phosphorus than did those from the nonfertilized plots. The small plants at Clemson were significantly higher in calcium than the large plants, but there was no significant difference in their phosphorus content.

The data from Lubbock were not included in the statistical analysis since there was only 1 composite sample from the 10 plots for each of the 2 years during which the turnip greens were grown. The results for both calcium and phosphorus were, however, comparable to the average results from other places.

FACTORIAL EXPERIMENTS

The analysis of variance (*11*) was applied to the data from the 30 factorial experiments. The interaction of block $\times$ treatments was the error item used. The method devised by Yates (*30*) for the analysis of factorial experiments was used to determine the effects of the fertilizer treatments. It will be noted that in the tables of factorial experiments, both simple averages and effects are given. These effects are the answers to algebraic equations in which the entire set of data is used for each effect (*23*). In each case the effects are for 8 times as many observations as the simple averages. Consequently, where apparent conflicts occur, more reliance can be placed upon the effects than upon the simple averages. Main effects, i. e., those of N, P, K, and Ca, correspond quite well with the treatments, but a word of caution is necessary in connection with the interactions. For instance, in the summation of 29 experiments given in table 7, both N and P treatments give a decrease in calcium content in comparison with the check. The effects are negative. If N and P give a decrease in calcium content, then it is to be expected that the combination NP would give a decrease. This is shown to be true in case of the N, P, and NP treatments, and the value of -0.037 for the interaction effect of NP is in addition to the decreasing effect shown by N and by P as main effects. (Interactions between 2 factors are called first-order interactions; between 3, second-order; and between 4, third-order. The most emphasis is placed on first-order interactions, since those of higher order are difficult to interpret. With one exception the latter, therefore, are not discussed).

Twenty-nine of the experiments were combined for a study of the total effects. Chi-square tests were applied and the experiments grouped according to their homogeneity. The low-error groups were used to study the causes of variability in the calcium and phosphorus content of the greens, because those groups included the majority of the experiments and because the variation not accounted for was smallest in these groups. The 29 experiments and the low-error groups were divided into subgroups for the purpose of studying the relations between the soil constituents and fertilizer treatments as they influence the mineral content of the greens.

CALCIUM

The average calcium content of the turnip greens from duplicate plots, the mean percent, and the standard deviation for each experiment are given in table 5. The range for the mean percent of calcium in the 30 experiments was from 1.74 to 5.076. The greens grown at

TABLE 5.—*Calcium content of turnip greens grown by a factorial design of 16 duplicate fertilizer treatments in 30 experiments*

Experiment No.	Location	Year	Average content of calcium (dry basis) from duplicate plots fertilized with—																Mean	Standard deviation	Odds of—	
			NPKCa	NPK	NPCa	NP	NKCa	NK	NCa	N	PKCa	PK	PCa	P	KCa	K	Ca	Check			19:1	99:1
			Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.			
	Georgia:																					
1	Blairsville	1939	2.035	2.110	2.120	2.060	2.525	2.690	2.480	2.465	3.535	4.375	4.170	4.240	4.570	3.590	3.615	4.085	3.167	0.422	0.899	1.242
2	----do----	1940	2.590	2.360	2.935	2.650	3.015	3.115	3.540	3.325	3.265	2.975	2.940	2.930	3.225	3.370	3.125	3.160	3.033	.187	.399	.552
3	Experiment	1939	2.295	2.130	2.165	2.380	2.060	2.030	2.210	1.965	3.020	2.805	2.730	2.635	2.860	2.930	2.715	2.545	2.467	.392	.834	1.154
4	----do----	1940 [1]	2.800	2.780	2.740	2.835	2.670	2.665	2.835	2.610	2.995	2.850	2.930	2.950	2.925	3.090	2.730	2.705	2.819	.098	.209	.288
5	Tifton	1939	2.230	2.170	3.005	3.045	2.715	2.460	3.270	3.045	2.805	2.655	3.035	2.635	2.630	2.615	2.835	2.475	2.727	.199	.424	.588
	Mississippi:																					
6	Crystal Springs	1939 [1]	1.855	1.760	1.940	1.715	1.815	1.770	1.840	1.655	2.245	2.130	2.150	2.255	2.330	2.205	2.105	2.185	1.997	.144	.305	.424
7	----do----	1940	1.530	1.680	1.490	1.515	1.540	1.660	1.555	1.740	2.730	2.510	2.705	2.725	2.630	2.755	1.610	2.680	2.128	.197	.421	.583
8	Natchez	1939	2.655	2.935	2.780	2.790	2.880	2.820	2.690	2.785	3.385	3.125	3.435	3.720	3.565	3.075	3.395	3.095	3.071	.327	.662	.914
9	Poplarville	1939	2.180	1.890	2.470	2.330	2.180	1.995	2.460	2.335	2.610	2.495	2.605	2.285	2.500	2.930	3.260	2.510	2.402	.310	.696	.964
10	----do----	1939 [1]	2.005	1.975	2.090	1.930	1.980	1.960	2.010	2.010	2.320	2.215	2.280	2.245	2.310	2.280	2.130	2.245	2.124	.126	.269	.371
11	----do----	1940	1.790	1.765	1.880	1.805	1.835	1.940	1.765	1.755	2.525	2.160	2.750	2.170	2.480	2.715	2.195	2.195	1.992	.209	.444	.614
12	Stoneville	1939 [1]	2.660	2.795	2.780	2.995	3.020	2.820	3.005	2.920	2.645	2.665	2.750	3.100	2.805	2.715	2.705	2.865	2.828	.268	.571	.789
13	----do----	1940	2.520	2.695	2.145	2.560	2.570	2.555	2.495	2.400	3.625	3.335	3.385	3.130	3.245	3.200	3.070	3.080	2.876	.113	.240	.334
14	West Point	1939	4.745	4.380	4.880	4.805	4.915	5.000	5.175	5.215	5.365	5.045	4.980	4.885	5.520	5.375	5.545	5.380	5.076	.178	.385	.523
	Oklahoma:																					
15	Heavener	1939	3.152	3.214	3.400	3.212	3.338	3.343	3.342	3.532	3.697	3.737	3.886	3.798	3.656	3.800	3.566	3.550	3.514	.117	.249	.348
16	Perkins	1939	2.265	2.730	2.475	2.635	3.035	2.420	2.615	2.305	2.985	3.080	2.665	2.940	2.915	2.445	2.920	2.435	2.674	.346	.738	1.021
17	----do----	1940	2.665	2.810	2.975	2.920	2.840	2.940	2.870	2.905	3.070	3.145	3.125	3.331	3.035	3.095	3.035	2.990	2.967	.160	.342	.472
18	Woodward	1939	2.876	2.882	2.771	2.903	3.056	2.831	2.904	3.015	3.390	3.361	3.335	3.384	3.736	3.616	3.236	3.430	3.167	.191	.410	.569
19	----do [2]----	1939	3.240	2.903	3.205	3.265	3.215	3.191	3.190	2.890	3.270	3.249	3.443	3.487	3.322	3.159	3.398	3.525	3.240	.170	.362	.501
20	----do [2]----	1940	3.296	3.280	3.272	3.158	3.316	3.169	3.361	3.313	3.822	3.780	3.609		3.692	3.511	3.604	3.671	3.459	.137	.291	.404
	South Carolina:																					
21	Clemson	1939 [1]	3.155	3.200	2.970	3.110	3.040	3.015	2.835	2.980	3.300	3.415	3.040	3.025	3.205	3.390	3.170	3.095	3.121	.147	.314	.430
22	Edisto	1939 [1]	2.750	2.175	2.420	2.535	2.525	2.485	2.855	2.595	3.030	2.605	2.430	2.370	2.805	2.710	2.795	2.575	2.604	.181	.387	.535
23	Sandhill	1940	1.550	1.385	1.390	1.545	1.785	2.195	1.615	1.465	1.895	2.060	1.605	1.585	2.035	2.005	1.765	1.955	1.740	.204	.436	.602
	Texas:																					
24	College Station	1940 [1]	2.410	2.440	2.145	2.535	2.225	2.490	2.375	2.370	2.640	2.640	2.915	2.710	2.550	2.670	2.650	2.605	2.527	.148	.314	.436
25	Iowa Park [3]	1939 [1]	2.428	2.345	2.515	2.562	2.343	2.689	2.048	2.168	3.019	2.724	2.726	2.696	2.194	2.308	2.526	2.458	2.484	.251	.758	1.047
26	Winter Haven	1939 [1]	2.294	2.209	2.256	2.192	2.314	2.135	2.320	2.237	3.508	2.349	2.490	2.419	2.498	2.343	2.495	2.322	2.336	.152	.325	.450
27	----do----	1940 [1]	2.275	2.145	2.085	1.975	2.145	1.960	2.035	2.190	2.275	2.510	2.235	2.245	2.420	2.345	2.315	2.260	2.213	.229	.489	.676
	Virginia:																					
28	Blacksburg	1940	3.260	2.690	3.400	2.900	3.210	2.750	3.430	2.750	3.250	3.170	3.295	3.180	3.525	2.940	3.075	3.095	3.120	.240	.512	.707
29	Norfolk	1939 [1]	2.305	2.025	1.785	2.105	2.375	1.865	2.355	2.065	2.550	2.160	2.120	2.605	2.360	2.240	2.560	3.080	2.285	.425	.905	1.253
30	----do----	1940 [1]	2.625	2.455	2.740	2.680	2.690	1.795	2.875	2.565	2.715	2.530	2.770	2.745	2.740	2.745	2.895	2.740	2.707	.141	.303	.416

[1] Fall crops. Undesignated crops were grown in the spring.
[2] Plots were irrigated.
[3] Iowa Park plots were not replicated; hence the mean is an average of 16 plots.
[4] Calculated result for 1 missing plot.

Sandhill contained the least calcium and those at West Point the most. The significant effects of each of the 16 fertilizer treatments for all experiments are shown in table 6. The effects were compared on the basis of standard error of the mean. Thirteen treatments had a significant effect upon the calcium content of the turnip greens in 1 or more experiments. There were no significant interaction effects for the treatments NKCa and KCa in any experiment. There were no fertilizer treatments that gave a significant effect at Stoneville, fall of 1939, and Norfolk, fall of 1940. It is interesting to note, however, that at Stoneville, spring of 1940, 6 treatments gave significant effects. This is the largest number of significant effects for any one place.

Of the 4 fertilizer factors studied, nitrogen had the greatest effect. It decreased the calcium content of the greens in all experiments except those at Stoneville, fall of 1939, and at Tifton. The decrease was significant in 24 of the 30 experiments. The average depression of calcium by nitrogen was approximately 0.36 percent (table 7). Applied calcium significantly increased the calcium content of the greens in 4 of the 30 experiments; namely, at Poplarville, spring of 1939; Edisto, fall of 1939; Winter Haven, fall of 1939; and Blacksburg, spring of 1940. The effect for calcium was positive in 20 experiments, with an average increase of 0.060 percent (table 7). There was a tendency for applied phosphorus to decrease the calcium content of the greens, the average decrease being 0.046 percent (table 7). The decrease was, however, significant only at Blairsville, spring of 1940, and at West Point and Sandhill. At Experiment, 1940, Stoneville, 1940, and Iowa Park a significant increase in calcium resulted from the application of phosphorus. The application of potassium produced a significant increase in calcium at Stoneville, 1940, Clemson, 1939, and Sandhill; and a significant decrease at Tifton and at Poplarville, spring of 1939. The interaction effect of NK was significantly negative at Blairsville, 1940, Experiment, 1940, Tifton, West Point, and Edisto. There was also a significant negative interaction effect of NP at Blairsville, 1940, Tifton, Stoneville, 1940, and Heavener. The treatment PK had a significant positive interaction effect at Woodward (irrigated plot) and at Edisto.

The average calcium content of the greens, the effects for 29 experiments combined and for the low- and high-error groups, and the F values from analyses of variance, are given in table 7. The data from Iowa Park were omitted because the fertilizer treatments were not replicated at this place. For the combined data, and for the low-error group, 5 treatments gave significant effects which were the same: N and P gave a highly significant negative effect, and Ca a highly significant positive effect, while NP and NK showed a significant negative interaction effect. There was a significant interaction of treatment $\times$ places for N, P, K, Ca, NP and NK in the 29 experiments and in the low-error group. This shows that the effects of the individual fertilizer treatments varied with the place. In the high-error group, N gave a highly significant negative effect and a significant interaction treatment $\times$ places as in the other 2 groups. There was also a significant negative interaction effect for PCa.

The F values in the analyses of variance were determined both on the basis of error variance and on the basis of interaction variance

TABLE 6.—*Significant effects of the 16 fertilizer treatments on calcium content of turnip greens from the 30 experiments*

Experiment No.	Location	NPKCa	NPK	NPCa	NP	NKCa	NK	NCa	N	PKCa	PK	PCa	P	KCa	K	Ca	19:1	99:1
	Average effect of fertilizer treatments on the percentage of calcium																**Odds of —**	
1	Georgia: Blairsville								−1.712**								1.318	0.439
2	do.				−0.211**		−0.256**		−.183*								.141	.195
3	Experiment		+0.091*						−.626***				−0.404**				.295	.408
4	do.				−.202*		−.081*		−.155***				+.081*				.074	.102
5	Tifton						−.314***			+0.086*					−0.383**		.150	.208
6	Mississippi: Crystal Springs								−.407**								.108	.150
7	do.								−1.079**								.149	.206
8	Natchez								−.558***								.231	.323
9	Poplarville								−.344**						−.250*	+0.233*	.246	.341
10	do.								−.258**								.095	.131
11	do.								−.400**								.157	.217
12	Stoneville								−.767***								.202	.279
13	do.			−.152**	−.122**		−.194**	−0.132**					+.097*		+.184**		.085	.118
14	West Point								−.373***				−.380**				.136	.185
15	Oklahoma: Heavener				−.140**				−.305**			−0.350*					.088	.123
16	Perkins								−.203**								.261	.361
17	do.																.121	.107
18	Woodward								−.525**								.145	.201
19	do.								−.206**								.128	.177
20	do.								−.376**		+0.114*						.103	.143
21	South Carolina: Clemson								−.142*	+.175*					+.162**		.111	.153
22	Edisto						−.181*				+.185*					+.195**	.137	.189
23	Sandhill	+0.161*	−.168*						−.247***				−.226**		+.248**		.154	.213
24	Texas: College Station								−.306**								.111	.154
25	Iowa Park												+.285*				.208	.370
26	Winter Haven								−.183**							+.121*	.115	.159
27	do.								−.224*								.173	.239
28	Virginia: Blacksburg								−.221*							+.450**	.181	.250
29	Norfolk								−.349***								.320	.443
30	do.																.107	.147

* = Significant at odds of 19:1; ** = significant at odds of 99:1.

(14). The effects of each of the variables, place, treatment, and replication, were highly significant for the low- and high-error groups, as well as for the combined experiments. Place (experiment) had much the greatest effect, as shown by the F values. These values were 12 to 13 times greater for place than for treatment in the low-error group. Replications had the smallest effect. There was a highly significant interaction between place and treatment in both the low- and high-error groups and for the 29 experiments combined.

TABLE 7.—*Results of 29 experiments combined and of those in the low- and high-error groups, showing the average calcium content of turnip greens, the average effects of the fertilizer treatments, and the F values from the variance analysis of each group—*

Fertilizer treatment	Total group (29 experiments [1])		Low-error group (22 experiments [2])		High-error group (7 experiments [3])	
	Average Ca content, 58 plots (dry basis)	Average effect of treatment, 464 plots	Average Ca content, 44 plots (dry basis)	Average effect of treatment, 352 plots	Average Ca content, 14 plots (dry basis)	Average effect of treatment, 112 plots
	Percent		*Percent*		*Percent*	
NPKCa	2.552	+0.010	2.619	+0.010	2.342	+0.011
NPK	2.482	−.012	2.516	−.008	2.374	−.024
NPCa	2.576	−.003	2.642	−.006	2.368	+.007
NP	2.589	−.037*	2.627	−.030*†	2.471	−.059
NKCa	2.649	−.012	2.671	−.007	2.582	−.031
NK	2.582	−.044**†	2.647	−.062**††	2.377	+.013
NCa	2.700	−.004	2.750	−.003	2.545	−.004
N	2.600	−.363**††	2.662	−.309**††	2.406	−.532**††
PKCa	3.016	+.019	3.033	+.027	2.961	−.009
PK	2.929	−.004	2.919	−.002	2.958	−.009
PCa	2.924	+.025	2.924	+.008	2.925	−.127*
P	2.929	−.046**††	2.882	−.051**††	3.075	−.030
KCa	3.031	−.025	3.018	+.012	3.071	+.065
K	2.916	−.006††	2.965	+.005††	2.760	−.042
Ca	2.972	+.060**††	2.956	+.061**††	3.024	+.057
Check	2.916		2.907		2.945	
Mean	2.773		2.796		2.699	
Standard deviation	.229		.167		.360	
Odds at 19:1	.091	.032	.076	.027	.291	.103
Odds at 99:1	.124	.044	.105	.037	.402	.142
Treatment F values:						
Residual as error	41.97**		45.18**		9.06**	
Place × treatment as error	17.10**		15.28**		4.29**	
Place F values:						
Residual as error	250.09**		586.26**		28.27**	
Place × treatment as error	101.89**		198.25**		13.39**	
Replication F value:						
Residual as error	2.19**		1.92**		2.37*	

[1] Iowa Park is omitted from the variance analyses.
[2] Factorial experiment Nos. 2, 4, 5, 6, 7, 10, 11, 13, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 30.
[3] Factorial experiment Nos. 1, 3, 8, 9, 12, 16, 29.
†=significant interaction of treatment × places at odds of 19:1; ††=significant interaction of treatment × places at odds of 99:1.
*=Significant at odds of 19:1; **=significant at odds of 99:1. †=significant interaction of treatment × places at odds of 19:1; ††=significant interaction of treatment × places at odds of 99:1.

THE EFFECTS OF FERTILIZER TREATMENT AS RELATED TO LEVEL OF SOIL CALCIUM

It seemed probable that the calcium content of the turnip greens was influenced not only by the fertilizer treatments, but also by the interrelated effect of the treatments and the soil calcium. The effects of the fertilizer treatments at different levels of soil calcium were, therefore, studied. The soils were classified as trace-, low-, medium-, and high-calcium soils (table 2) and the effects of the fertilizer treatments determined for each class in the 28 experiments from which

soil samples were analyzed, and in the 22 experiments of the low-error group. The results for the low-error group and the F values for the analyses of variance are shown in table 8. There was a significant increase in the calcium content of the greens when calcium was added to the soil in the trace group, and a small but significant increase when it was added to the soil in the low- and high-calcium groups. The greatest effect for calcium treatment was shown in the trace-calcium

TABLE 8.—*Results of the experiments from the low-error group, showing the calcium content of turnip greens grown on trace-, low-, medium-, and high-calcium soils, the average effect of the fertilizer treatments, and the F values from the variance analysis of each group*

Fertilizer treatment	Trace-calcium soils (5 experiments[1]) Average Ca content, 10 plots (dry basis)	Average effect of treatment, 80 plots	Low-calcium soils (4 experiments[2]) Average Ca content, 8 plots (dry basis)	Average effect of treatment, 64 plots	Medium-calcium soils (3 experiments[3]) Average Ca content, 6 plots (dry basis)	Average effect of treatment, 48 plots	High-calcium soils (10 experiments[4]) Average Ca content, 20 plots (dry basis)	Average effect of treatment, 160 plots
	Percent		*Percent*		*Percent*		*Percent*	
NPKCa	2.167	+0.025	2.234	−0.003	2.742	−0.006	2.962	+0.013
NPK	1.997	−.069*	2.099	+.037	2.821	+.029	2.851	−.007
NPCa	2.353	+.027	2.148	−.033	2.840	−.011	2.926	−.014
NP	2.245	−.032	2.150	−.002	2.889	−.089**	2.930	−.022
NKCa	2.305	−.028	2.138	−.025	2.801	+.025	3.028	+.002
NK	2.221	−.103**†	2.145	−.064*	2.924	−.011	2.976	−.056**††
NCa	2.418	+.009	2.271	+.004	2.862	−.029	3.073	−.005
N	2.205	−.219**††	2.150	−.441**††	2.936	−.301**†	3.013	−.304**††
PKCa	2.559	+.023	2.750	+.069*	3.136	+.010	3.353	+.018
PK	2.452	−.015	2.524	+.007	3.174	−.052	3.235	+.017
PCa	2.392	−.031	2.554	+.027	3.309	+.028	3.223	+.013
P	2.363	−.065*	2.575	−.016	3.238	+.032	3.158	−.084**††
KCa	2.596	+.012	2.673	+.027	3.040	−.045	3.360	+.025
K	2.371	−.030†	2.690	+.038	3.188	−.049	3.305	+.025††
Ca	2.536	+.135**††	2.560	+.057*††	3.084	−.052	3.285	+.059**
Check	2.393	----	2.536	----	3.058	----	3.267	----
Mean	2.348		2.387		3.003		3.122	
Standard deviation	.199		.160		.143		.164	
Odds 19:1	.178	.063	.158	.056	.167	.059	.102	.036
Odds 99:1	.235	.083	.212	.075	.221	.078	.136	.048
Treatment F values:								
Residual as error	6.19**		20.14**		8.81**		216.28**	
Place×treatment as error	2.26*		3.32*		7.85**		8.05**	
Place F values:								
Residual as error	273.14**		189.38**		383.36**		747.00**	
Place×treatment as error	99.72**		35.40**		343.00**		278.05**	
Replication F value:								
Residual as error			1.05				2.60**	

[1] Factorial experiment Nos. 5, 10, 11, 23, 28.
[2] Factorial experiment Nos. 4, 6, 7, 22.
[3] Factorial experiment Nos. 15, 17, 24.
[4] Factorial experiment Nos. 2, 13, 14, 18, 19, 20, 21, 26, 27, 30.
*=Significant at odds of 19: 1; **=significant at odds of 99: 1; †=significant interaction of treatment × places at odds of 19: 1; ††=significant interaction of treatment×places at odds of 99:1.

soils. The medium group did not show any effect from applied calcium, but this may have been due to an insufficient number of experiments. The average percentage of calcium in the greens increased as the calcium content of the soil increased, the average for the greens grown on the high-calcium soils being 33 percent greater than that for the greens grown on the trace-calcium soils. Nitrogen significantly decreased the calcium content of the greens in all groups.

Phosphorus also had a negative effect in the trace- and high-calcium soils. NK showed a significant negative interaction effect in the trace-, low-, and high-calcium soils, and NP in the medium-calcium soils.

The effect of the treatments on the different soils within a group was not uniform as is shown by the interaction between treatment and places. There was a significant interaction, treatment x places, for N in all groups; for Ca in the trace- and low-calcium soils; for P in the high-calcium soils; and for K in the trace- and high-calcium soils.

The results both for the average percentage calcium and for effects were essentially the same for the total of 28 experiments[7] as for the 22 experiments in the low-error group (table 8). There was, however, a highly significant interaction between treatment and places for NP in the high-calcium soils of the total experiments.

In the variance analyses of the low-error experiments, the F values for treatment determined on the basis of treatments $\times$ places interaction, variance increased as the calcium content of the soil increased. These values in the trace-, low-, medium-, and high-calcium soils were 2.26, 3.32, 7.85, and 8.05, respectively. In the low-, medium-, and high-calcium soils of the 28 experiments,[7] the F values followed the same trend.

Effect of the Level of Organic Matter in the Soil

The effect of the level of organic matter in the soil on the calcium content of turnip greens was also studied in connection with the fertilizer treatments. The results of 21 experiments in the low-error group and the F value for analyses of variance are shown in table 9. The soils were divided, according to the amount of organic matter present, into three groups which for convenience will be referred to as a low, medium, and high group, containing 0.48 to 1.20, 1.20 to 2.00, and 2.00 to 4.00 percent organic matter, respectively. The division was based upon the fact that the soils fell naturally into these three groups, there being an interval between each group. Experiment 14 (West Point) of the low-error group, was eliminated because it introduced heterogeneity into the organic-matter grouping. This soil contained almost three times as much exchangeable calcium as that of any other soil. Correlation studies showed that exchangeable calcium in the soil had a greater influence on the calcium content of the greens than did organic matter.

It will be seen from table 9 that the mean calcium in the greens increased with an increase in organic matter. Applied calcium significantly increased the calcium content of the greens in the medium and high organic-matter groups, but the effect was greater in the group containing the most organic matter. Nitrogen significantly decreased the calcium in all three organic-matter groups.

Since it seemed possible that the results secured for the effect of organic matter might depend upon the level of soil calcium, the soils were subdivided into a high- and a low-calcium group and similarly studied in order to compare the main effects with those shown in table 9. The most important results were those for the mean calcium in the

[7] Table not shown, but is available at the Mississippi Experiment Station.

greens and the effects of applied calcium and nitrogen.   In the high- and low-calcium soils there was also an increase in the mean calcium in the greens with increased organic matter.   The effects of applied calcium and nitrogen correspond to those shown in table 9, but the effect of nitrogen was greater in the high- than in the low-calcium soils.

TABLE 9.—*Results of the experiments from the low-error group, showing the calcium content of turnip greens grown on soils containing different percentages of organic matter, the average effect of each fertilizer treatment, and the F values from the variance analysis of each group*

Fertilizer treatment	Soils containing 0.48 to 1.20 percent organic matter (7 experiments[1])		Soils containing 1.20 to 2.00 percent organic matter (11 experiments[2])		Soils containing 2.00 to 4.00 percent organic matter (3 experiments[3])	
	Average Ca content, 14 plots (dry basis)	Average effect of treatment, 112 plots	Average Ca content, 22 plots (dry basis)	Average effect of treatment, 176 plots	Average Ca content, 6 plots (dry basis)	Average effect of treatment, 48 plots
	Percent		*Percent*		*Percent*	
NPKCa	2.334	+0.009	2.550	+0.010	2.825	+0.016
NPK	2.303	−.013	2.486	+.006	2.502	−.026
NPCa	2.280	+.013	2.565	−.035*	3.025	+.034
NP	2.302	−.009	2.604	−.040*	2.743	−.053†
NKCa	2.335	−.002	2.598	−.002	2.972	−.039
NK	2.386	−.034	2.533	−.061**††	2.887	−.088*††
NCa	2.282	−.012	2.681	−.010	3.282	+.059
N	2.329	−.282**††	2.582	−.363**††	2.880	−.154**
PKCa	2.665	+.005	3.044	+.024	3.077	+.074
PK	2.648	−.001	2.906	−.006	2.892	−.008
PCa	2.529	+.014	2.967	−.000	3.002	−.004
P	2.540	−.020†	2.899	−.007	2.952	−.178**††
KCa	2.665	+.014	2.975	+.019	3.163	−.030
K	2.644	+.075**††	2.936	−.006††	3.018	−.099*
Ca	2.549	−.010	2.901	+.058**†	3.242	+.214**††
Check	2.566		2.874		2.998	
Mean	2.460		2.756		2.966	
Standard deviation	.172		.159		.193	
Odds at 19:1	.130	.046	.093	.033	.223	.079
Odds at 99:1	.170	.060	.124	.044	.300	.106
Treatment *F* values:						
Residual as error	11.06**		33.13**		5.79**	
Place × treatment as error	7.51**		8.55**		2.55*	
Place *F* values:						
Residual as error	441.02**		269.43**		46.80**	
Place × treatment as error	299.41**		69.49**		20.64**	
Replication *F* values:						
Residual as error	.69		2.04*		2.47	

[1] Factorial experiment Nos. 10, 11, 15, 21, 23, 24, 27.
[2] Factorial experiment Nos. 4, 5, 6, 7, 13, 17, 18, 19, 20, 22, 26.
[3] Factorial experiment Nos. 2, 28, 30.
*= Significant at odds of 19 : 1; **= significant at odds of 99 : 1; †= significant interaction of treatment × places at odds of 19 : 1; ††= significant interaction of treatment × places at odds of 99 : 1.

Other significant effects shown in table 9 are those for K, P, NP, and NK.   Potassium increased the calcium content of the greens in the low organic-matter soils, but decreased it in the high organic-matter soils. The effect of phosphorus was to decrease the calcium in the high organic-matter group.   NP and NK produced a significant negative interaction effect in the medium organic-matter group, and NK in the high group as well.   In the majority of instances, there was a significant interaction between treatment and places for those treatments which showed significant effects.   Exceptions to this were K in the medium, and P in the low organic-matter soils where there were interactions of treatment × places, but no significant effects.

When the 28 experiments [7] were studied with respect to organic matter as were those in the low-error group, the effects and interactions of treatment × places were similar. K, however, failed to show a significant effect in any group, whereas P gave a significant negative effect in the medium as well as in the high organic-matter group. PCa had a significant negative interaction effect, and KCa a significant positive effect in the medium organic-matter soils.

The F values from analyses of variance showed that there was a highly significant variation for both place and treatment. The variation due to place decreased with increased organic matter. The above was true for both the low-error group and the 28 experiments.

Correlation Coefficients and Regression Equations

By means of correlation coefficients and regression equations, it was possible to study the relation between the calcium content of the greens and a number of soil properties. These included the available soil calcium as determined by the rapid soil test, exchangeable calcium total exchange capacity, percent organic matter, and soil pH. Ex-

Table 10.—*Correlation coefficients and regression equations showing relations between exchangeable calcium and percentage of organic matter, and the calcium in the greens, for the mean and for plots showing significant places × treatment interactions*

Plot	*r*		R (exchangeable calcium and organic matter)	Regression equations [1]	Standard error of estimate
	Exchangeable calcium	Organic matter			
NP	0.4696*	0.0998	0.4729**	$2.0995+0.1086x+0.0343x_1$	0.2976
NK	.5277**	.2115	.5523**	$1.9708+.1131x+.0938x_1$	.4307
N	.4461*	.1926	.4712**	$2.0092+.1043x+.0955x_1$	.4997
P	.4667*	.3399	.5535**	$2.1596+.1099x+.1945x_1$	.3918
K	.4477*	.3253	.5306**	$2.2304+.0970x+.1712x_1$	.4572
Ca	.4034*	.3478	.5097**	$2.2993+.0835x+.1813x_1$	.4494
Mean of 16 replicated treatments in each locality	.5257**	.2861	.5771**	$2.1237+.1064x+.1313x_1$	.4041

[1] x=Exchangeable calcium; x_1=organic matter.
*=Significant at odds of 19:1; **=significant at odds of 99:1.

changeable calcium gave the best basis for prediction, and percent organic matter was less related to it than were the other properties. Accordingly, correlation coefficients and regression equations were determined for exchangeable calcium and organic matter in the NP, NK, N, PK, and Ca plots, and for the mean of the experiments, omitting West Point. These plots were studied because the effects of treatment varied greatly at different places and could not be described satisfactorily by the mean regression. The results are given in table 10. The simple correlation coefficients showed a significant positive relation between the calcium in the greens and exchangeable calcium, and a positive relation which was not significant between the calcium in the greens and organic matter. The multiple correlation coefficients for exchangeable calcium and organic matter show a somewhat closer relation than the simple correlation coefficients.

Since the rapid soil test for available calcium, although not quantita-

[7] Table now shown, but is available at the Mississippi Experiment Station.

tive, has the advantage of speed and simplicity, it seemed worth-while to determine the relation between the calcium in the greens and the level of available soil calcium. The plots studied were the check plots and those to which nitrogen and phosphorus had been added, as the majority of soils were low in these constituents. The correlation coefficients and regression equations are given in table 11. The correlation coefficients show that there was a significant relation between the available soil calcium and that of the greens. The standard error of estimate indicates that the regression equation for the NPK plots is a fairly accurate means for predicting the calcium content of the plant when the rapid soil test for available calcium is used.

TABLE 11.—*Correlation coefficients and regression equations showing relations between available soil calcium and the calcium in the greens on check and fertilized plots*

Plot	r (available soil calcium)	Regression equation[1]	Standard error of estimate
NPKCa	0.5354**	1.8771+0.0954X	0.5640
NPK	.5702**	1.7282+ .1048X	.3829
NPCa	.4419*	1.9645+ .0859X	.6531
NP	.4894**	1.9494+ .0900X	.6006
Check	.5300**	2.0352+ .0888X	.6407

[1] X-available soil calcium. Levels of available soil calcium ranging from $T-$ to $H+$ (table 2) were arbitrarily assigned values from 1 through 12.

COVARIANCE ANALYSES

Covariance analyses were made on the data secured from the 9 places in which crops were grown in 2 successive years. When the means for the calcium content of the greens were adjusted on the basis of soil calcium, as determined by rapid soil tests, the significance of the variation due to place was not eliminated, but when this was done for exchangeable calcium the variation due to place was eliminated.[7] The unadjusted F value for the 9 places was small in comparison with the corresponding F value for the 28 experiments. Hence, itseemed likely that the number of cases was insufficient.

The covariance analyses, using the 2 series instead of successive crops, were, therefore, worked out for the check and NPK plots in the 28 experiments. It was thought that the calcium in the greens from the check plots would reflect the effects of the soil since no fertilizer was added. The NPK plots, however (to which no calcium was applied), would produce more normal growth. Table 12 shows the relation of the multiple variates, organic matter and exchangable calcium, to the variation in the calcium content of the greens which was attributed to place. Only the maximum possibility for the effects of the soil properties on the variation due to place could be studied since there was but one soil analysis for both series. The F value for place in the 28 experiments was 15.91**. This value was reduced to 6.06** when the means were adjusted to eliminate the effect of exchangeable calcium. Since the exchangeable calcium of the West Point soil was so much greater than that of any other soil, an analysis of 27 experiments, excluding West Point, was made.[7] The

[7] Table not shown, but is available at the Mississippi Experiment Station.

TABLE 12.—*Covariance analyses of the average calcium content of turnip greens from the check and NPK plots, showing the effect of exchangeable calcium and organic matter in variation due to place*

Plot	Source of variation	Variance of calcium content of greens (y)				Error of estimate based on exchangeable calcium (x)				Error of estimate based on exchangeable calcium and percent of organic matter				
		Degrees of freedom	Sy^2	Mean square	F	Degrees of freedom	$Sy^2 - \frac{(Sxy)^2}{Sx^2}$	Mean square	F	Degrees of freedom	R^2	$(1-R^2)Sy^2$	Mean square	F
Check	Total	55	28.8846			54	12.4683			53	0.6458	10.2239		
	Place	27	27.0992	1.0037	15.91**									
	Error	28	1.7654	.0631		27	1.7654	.0654		26	0	1.7654	0.0679	
	Difference for testing adjusted means					27	10.7029	.3964	6.06**	27		8.4585	.3133	4.61**
NPK	Total	53	14.2900			52	10.4763			51	.5420	6.5448		
	Place	26	13.2451	.5094	13.16**									
	Error	27	1.0449	.0387		26	1.0449	.0402		25	0	1.0449	.0418	
	Difference for testing adjusted means					26	9.4314	.3627	9.02**	26		5.4999	.2115	5.06**

**=Significant at odds of 99 : 1.

F value for place was 8.64**, as compared to 15.91**, indicating that a large portion of the variation attributed to place was accounted for by conditions associated with the West Point experiment. The *F* value for the adjusted means, however, was 5.97**—a value almost identical with the *F* value for the adjusted means of the 28 experiments. The *F* value for place in the 27 experiments was 4.61** when the effects of exchangeable calcium and organic matter were eliminated. For the 22 experiments in the low-error group, the *F* value for place was 20.05**.[7] This was reduced to 4.94** when the means were adjusted on the basis of exchangeable calcium and organic matter. Correlation coefficients indicated that the hydrogen-ion concentration of the soil did not have as important an effect on the calcium in the greens as did exchangeable calcium. This result was verified when the relation of hydrogen-ion concentration to variation in the plant calcium due to place was determined for the check plots. Elimination of the effect of hydrogen-ion concentration reduced the *F* value for place in the 28 experiments from 15.91** to 11.64** while elimination of the effect of exchangeable calcium reduced it to 6.06**.

In the NPK plots, the *F* value for place was 13.16** for 27 experiments. Adjusting the mean values for the calcium content of the turnip greens on the basis of exchangeable calcium reduced this *F* value to 9.02**. It is evident from the data presented that the variation in soil composition affects the calcium content of the greens, but that no one factor, or any of the combinations studied, accounts for all the significant differences associated with the individual places.

PHOSPHORUS

The data on phosphorus were studied in a manner similar to that for calcium. Table 13 shows the average phosphorus in the turnip greens grown in duplicate plots, the mean for each experiment, and the standard deviation. The mean percentage of phosphorus in the greens from the 30 experiments ranged from 0.3531 at West Point to 0.7203 at Stoneville. Table 14 shows the significant effects of the fertilizer treatments. It will be seen that each of the fertilizer treatments had a significant effect in one or more experiments. There were four experiments, however, in which there was no significant effect for any treatment. The experiments were those at Experiment, spring of 1939, Winter Haven, fall of 1939 and 1940, and Norfolk, fall of 1939.

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*Significant at odds of 19:1; **significant at odds of 99:1.
[7] Table not shown, but is available at the Mississippi Experiment Station.

TABLE 13.—*Phosphorus content of turnip greens grown by a factorial design of 16 duplicate fertilizer treatments in 30 experiments*

Experiment No.	Location	Year	Average content of phosphorus (dry basis) of greens from duplicate plots fertilized with—																Mean	Standard deviation	Odds of—	
			NPKCa	NPK	NPCa	NP	NKCa	NK	NCa	N	PKCa	PK	PCa	P	KCa	K	Ca	Check			19:1	99:1
			Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.			
1	Georgia: Blairsville	1939	0.7090	0.8045	0.8450	0.7080	0.6000	0.5365	0.6305	0.5830	0.6235	0.5160	0.5855	0.5500	0.4270	0.5380	0.5265	0.4330	0.6010	0.0765	0.1632	0.2257
2	do	1940	6190	5745	5505	5720	4150	4370	4435	4275	6445	6105	6290	6550	5635	5150	5405	5845	5488	0554	1182	1635
3	Experiment	1939	5800	5270	5790	5890	5550	5510	5000	5475	5680	5655	5695	5680	5840	5785	5590	6000	5653	0385	0820	1134
4	do	1940[1]	4460	4650	4905	4570	4190	4445	4190	3890	4920	4585	4760	4315	4485	4080	3950	4015	4401	0304	0648	0899
5	Tifton	1939	4850	6610	7265	5935	5105	5685	5800	5300	5185	5780	5595	5980	5135	5865	5030	5530	5684	0517	1100	1525
6	Mississippi: Crystal Springs	1939[1]	7850	6600	7550	7950	7500	7450	7400	7550	6050	5000	5900	5750	5950	5700	5750	5750	6663	0246	0523	0724
7	do	1940	5050	4550	5250	4350	5000	4200	4250	3550	5950	5200	6050	6150	5850	6000	5400	5850	5203	0506	1078	1485
8	Natchez	1939	5900	6050	6550	6450	5400	5250	5950	5500	5650	5050	5550	4850	4950	5800	3200	6400	5709	0475	1013	1400
9	Poplarville	1939	4600	5000	5650	5650	4150	4000	3800	4150	5750	5730	5750	6300	5450	5050	3700	5250	4919	0784	1672	2311
10	do	1939[1]	4500	4600	4350	4650	3900	4450	4400	4100	4150	4500	4250	4400	3750	3900	3700	4150	4188	0232	0495	0684
11	do	1940	5350	5850	6250	5500	4400	5500	4400	4850	6000	6050	5800	6350	5550	6150	5950	6200	5569	0382	0707	0073
12	Stoneville	1939[1]	6750	6850	7150	7600	6550	7150	7150	7200	7600	7250	7000	6750	7750	7550	7500	7450	7203	0347	0738	1024
13	do	1940	7150	7000	7600	7700	7000	7000	7200	6900	6450	7000	6650	7050	6750	6600	6750	6750	6972	0316	0673	0936
14	West Point	1939	3700	3350	3800	3500	3650	3650	3900	3900	3350	3500	3100	3050	3450	3600	3450	3550	3631	0167	0354	0492
15	Oklahoma: Heavener	1939	7105	6425	6380	6665	4940	5070	5495	5610	7110	7025	7080	7150	6440	5725	6125	6300	6290	0372	0795	1097
16	Perkins	1939	4915	5545	6290	5465	4830	4280	4805	4555	5955	5605	5595	5495	5330	4680	5230	4950	5158	0384	0817	1131
17	do	1940	5405	4370	4860	5215	4775	4090	4325	4335	6125	5675	5960	5290	4405	4535	4970	4065	4865	0570	1213	1680
18	Woodward	1939	5190	5155	4700	4990	3800	4090	3970	4025	5280	5495	4965	5540	4360	4265	4210	4065	4631	0295	0634	0880
19	do[2]	1940	5835	5700	5950	6135	5005	5130	5120	5005	5415	6020	5725	5575	5445	5320	5165	5255	5491	0317	0676	0933
20	do[2]	1940	6145	0845	6090	6815	5830	5485	5150	5520	5905	6000	5875	6730	5920	5765	6795	5990	6058	0321	0684	0945
21	South Carolina: Clemson	1939[1]	4635	5495	5430	5365	5290	4790	5325	4895	5470	4885	5470	5585	5445	5270	5275	5635	5296	0261	0557	0769
22	Edisto	1939[1]	5585	6050	3865	5815	5590	5250	5700	5110	6570	6175	6415	5980	6240	6040	6350	5995	5921	0269	0577	0795
23	Sandhill	1940	5720	6565	5885	5595	4935	4935	5625	5800	5260	4965	5965	6200	5380	5745	5115	4680	5523	0412	0880	1213
24	Texas: College Station	1940[1]	7055	7400	8230	8010	7190	6515	6825	7745	6630	7015	6490	6550	6745	7295	6970	6000	7042	0573	1222	1689
25	Iowa Park[3]	1939[1]	390	355	378	381	401	334	321	436	380	388	386	437	493	447	413	427	3979	0437	1318	1821
26	Winter Haven	1939[1]	6960	6290	6265	6595	6595	6290	6470	6040	6895	6780	6710	7015	6375	6685	6685	6578	6578	0356	0758	1049
27	do	1940[1]	6560	6470	6760	6395	6695[4]	6290	6165	6435	6745	6165	6775	6600	6570	6575	6630	6015	6484	0385	0820	1134
28	Virginia: Blacksburg	1940	6575	6135	7555	6735	5280	5130	5295	4560	7430	6200	6600	7030	5700	5850	5735	5295	6069	0751	1698	2215
29	Norfolk	1939[1]	5500	4650	6600	6200	4300	5900	5300	4850	4700	5700	5700	4750	5250	4500	4000	4200	5163	1057	2251	3114
30	do	1940[1]	5955	5875	4815	5495	4075	4820	4590	4590	5825	5065	5415	5680	5305	5380	5090	3745	5145	0468	0998	1380

[1] Fall crops. Undesignated crops were grown in the spring.
[2] Plots were irrigated.
[3] Iowa Park plots were not replicated; hence, the Mean is an average of 16 plots.
[4] Calculated result for 1 missing plot.

TABLE 14.—*Significant effects of the 16 fertilizer treatments on the phosphorus content of turnip greens from 30 experiments*

Experiment No.	Location	Average effect of indicated fertilizer treatment on the percentage of phosphorus							
		NPKCa	NPK	NPCa	NP	NKCa	NK	NCa	N
	Georgia:								
1	Blairsville	−0.0660*							+0.1525**
2	do								−.0870**
3	Experiment								
4	do								
5	Tifton					−0.0499*			
	Mississippi:								
6	Crystal Springs	+.0213*				+.0200*	−0.0188*		+.1638*
7	do								−.1356**
8	Natchez			−0.0419*	+0.0409*				
9	Poplarville								−.0788*
10	do								+.0175*
11	Stoneville	−.0313*			+.0563**				−.0875**
12	do						−.0406**		+.0306*
13	do								+.0444**
14	West Point						−.0188*	+0.0125*	+.0300**
	Oklahoma:								
15	Heavener								−.0658**
16	Perkins					+.0488*			−.0394*
17	do								−.0549*
18	Woodward								−.0283*
19	do								
20	do	−.0270*			+.0476**		+.0309*		
	South Carolina:								
21	Clemson			−.0298**		−.0261*			−.0126*
22	Edisto								−.0000**
23	Sandhill	−.0630*	+0.0704**						
	Texas:								
24	College Station						−.0541*		+.0659**
25	Iowa Park								−.0469*
26	Winter Haven								
27	do								
	Virginia:								
28	Blacksburg								
29	Norfolk		+.0408*						
30	do								

TABLE 14.—*Significant effects of the 16 fertilizer treatments on the phosphorus content of turnip greens from 30 experiments*—Continued

Experiment No.	Location	Average effect of indicated fertilizer treatment on the percentage of phosphorus							Odds of—	
		PKCa	PK	PCa	P	KCa	K	Ca	19:1	99:1
	Georgia:									
1	Blairsville				+0.1338**				0.0577	0.0798
2	do				+.1161**				.0418	.0578
3	Experiment								.0290	.0401
4	do				+.0490**				.0229	.0318
5	Tifton				+.0500*	−.0614**			.0389	.0539
	Mississippi:									
6	Crystal Springs		−.0456*						.0185	.0256
7	do				+.0381*			+0.0444*	.0381	.0525
8	Natchez				+.1125**				.0358	.0495
9	Poplarville				+.0475**				.0591	.0817
10	do				+.0650**			−.0200*	.0175	.0242
11	do								.0250	.0344
12	Stoneville								.0261	.0362
13	do								.0238	.0331
14	West Point				−.0225**				.0125	.0174
	Oklahoma:									
15	Heavener				+.1154**				.0281	.0388
16	Perkins				+.0651**				.0289	.0400
17	do				+.0996**			+.0500*	.0429	.0594
18	Woodward				+.1066**				.0224	.0311
19	do				+.0621**				.0239	.0330
20	do			−.0406**	+.0501**				.0242	.0334
	South Carolina:									
21	Clemson				+.0273*		−.0213*		.0197	.0272
22	Edisto				+.0493***			+.0288*	.0204	.0281
23	Sandhill								.0311	.0429
	Texas:									
24	College Station								.0432	.0597
25	Iowa Park								.0466	.0644
26	Winter Haven								.0268	.0371
27	do								.0290	.0401
	Virginia:									
28	Blacksburg				+.1427**				.0565	.0783
29	Norfolk								.0796	.1101
30	do	+0.0419*			+.0741**		+.0435*		.0353	.0488

*Significant at odds of 19:1; **significant at odds of 99:1.

The most outstanding effect of the fertilizer treatments was that of phosphorus. It increased the phosphorus content of the greens significantly in 18 experiments. The average increase was 0.0537 percent (table 15). The effect of nitrogen on the phosphorus content of turnip greens was similar to its effect on calcium, although less consistent. Nitrogen decreased the phosphorus significantly in 12 experiments, but had a negative effect in 18 experiments. Six places, however, showed a significant increase; namely, Blairsville, spring of 1939; Crystal Springs, fall of 1939; Poplarville, fall of 1939; Stoneville, spring of 1940; West Point, 1939; and College Station, fall of 1940. Calcium added as fertilizer showed a tendency to increase the phosphorus content of the greens, but this increase was significant at only three places, Crystal Springs 1940, Perkins 1940, and Edisto, and there was a significant decrease in phosphorus at Poplarville, fall of 1939. At Clemson, 1939, the phosphorus content

TABLE 15.—*Results of 29 experiments combined and of those in the low- and high-error groups, showing the average phosphorus content of turnip greens, the average effects of the fertilizer treatments, and the F values from the variance analysis of each group*

Fertilizer treatment	Total group (29 experiments [1])		Low-error group (18 experiments [2])		Medium-error group (7 experiments [3])		High-error group (4 experiments [4])	
	Average P content, 58 plots (dry basis)	Average effect of treatment, 464 plots	Average P content, 36 plots (dry basis)	Average effect of treatment, 288 plots	Average P content, 14 plots (dry basis)	Average effect of treatment, 112 plots	Average P content, 8 plots (dry basis)	Average effect of treatment, 64 plots
	Percent		*Percent*		*Percent*		*Percent*	
NPKCa	0.5808	+0.0007††	0.5793	−0.0012	0.5772	+0.0099	0.5941	−0.0070†
NPK	.5834	+.0001	.5821	−.0001††	.5800	+.0029	.5958	−.0040
NPCa	.6094	−.0030	.5889	−.0005	.6068	−.0095	.7064	−.0025
NP	.5986	+.0151**	.5963	+.0135**††	.5882	+.0195**†	.6266	+.0146
NKCa	.5268	−.0026	.5547	−.0015	.5185	+.0041†	.5058	−.0195
NK	.5162	−.0092*	.5290	−.0052	.4896	−.0088†	.5049	−.0282
NCa	.5315	+.0016	.5403	−.0018	.5168	+.0045	.5175	+.0119
N	.5226	−.0059††	.5381	−.0065*††	.5042	−.0228**††	.4848	+.0260††
PKCa	.5889	+.0003	.5826	+.0015	.5973	+.0005	.6029	−.0054
PK	.5754	−.0083*	.5753	−.0022	.5784	−.0092	.5763	−.0338*
PCa	.5858	+.0001	.5763	−.0040	.6036	+.0031	.5976	+.0136
P	.5857	+.0537**††	.5845	+.0377**††	.5864	+.0613**†	.5895	+.1129**
KCa	.5511	−.0003	.5613	+.0035	.5445	−.0054†	.5168	−.0088
K	.5526	−.0045	.5524	−.0045	.5718	−.0038	.5195	−.0055
Ca	.5401	+.0054	.5562	+.0012	.5474	+.0098†	.4550	+.0161
Check	.5373		.5519		.5348		.4761	
Mean	.5616		.5643		.5591		.5540	
Standard deviation	.0504		.0381		.0522		.0844	
Odds at 19:1	.0201	.0071	.0184	.0065	.0421	.0149	.0899	.0318
Odds at 99:1	.0277	.0098	.0263	.0093	.0583	.0206	.1244	.0440
Treatment *F* values:								
Residual as error	20.62**		11.48**		7.14**		5.02**	
Place × treatment as error	9.13**		4.49**		2.59**		3.21**	
Place *F* values:								
Residual as error	95.07**		221.40**		59.10**		15.40**	
Place × treatment as error	42.08**		86.69**		21.44**		9.84**	
Replication *F* values:								
Residual as error	1.78**		0.43		2.50*		2.53*	

[1] Iowa Park is omitted from the variance analyses.
[2] Factorial experiment Nos. 3, 4, 6, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 26, 27.
[3] Factorial experiment Nos. 2, 5, 7, 8, 17, 24, 30.
[4] Factorial experiment Nos. 1, 9, 28, 29.
*=Significant at odds of 19: 1; **=significant at odds of 99: 1; †=significant interaction of treatment × places at odds of 19: 1; †† significant interaction of treatment × places at odds of 99: 1.

of the greens was significantly decreased by potassium, and at Norfolk, 1940, it was significantly increased. There were significant positive interaction effects for NP at Natchez, Poplarville, 1940, and at Woodward, 1940. The treatment NK gave a significant negative interaction effect at Crystal Springs, 1940, Stoneville, 1939, West Point and College Station, and a significant positive effect for the irrigated plot at Woodward.

The results for the 29 experiments combined, for the low-medium- and high-error groups and the *F* values for the analyses of variance are given in table 15. Four treatments gave significant effects for the 29 experiments. Phosphorus showed a highly significant positive effect. NK and PK had a significant negative interaction effect, and NP a highly significant positive interaction effect. There was a significant interaction between places and the treatment of N, P, and of NPKCa in the 29 experiments.

In the low-error group, the effects of the treatments P and NP corresponded to those for the 29 experiments. Nitrogen, however, gave a significant negative effect, whereas NK and PK gave no significant interaction effects. The effects showing significance for the medium-error group were like those for the low-error group. Only two treatments, P and PK, gave a significant effect in the high-error group, P having a highly significant positive effect as in the other groups and in the combined experiments, and PK a negative interaction effect. There was significant treatment $\times$ places interactions for the following treatments in the three error groups: NPKCa, NPK, NP, N, and P in the low-error groups; NP, NKCa, NK, N, P, KCa, and Ca in the medium-error group; and NPKCa and N in the high-error group.

In the variance analyses the effects of the three variables, place, treatment, and replication, on the phosphorus content of the greens were similar to those for calcium. Again the effect of place was much greater than that for treatment, although treatment had a highly significant effect. Replications had the smallest effect and this effect was not significant in the low-error group.

Effect of Fertilizer Treatment as Related to Level of Soil Phosphorus

The phosphorus content of the greens was studied in relation to the level of soil phosphorus and fertilizer treatments in the same manner as was the calcium content of the greens in relation to the soil calcium. Table 16 shows the results for the low-error experiments and the *F* values for analyses of variance when the soils were grouped into low-, medium-, and high-phosphorus soils. The majority of the soils on which the turnip greens were grown were, however, low-phosphorus soils. Two of the three experiments having medium-phosphorus soils and both having high-phosphorus soils were in the low-error group. The high-phosphorus soils were in reality the same soil, since the two experiments were conducted at the same place, but in different seasons. The data are, therefore, insufficient in the medium- and high-phosphorus groups to do more than indicate trends.

It will be seen from the table that the mean phosphorus content of the greens increased as the soil phosphorus increased, the phosphorus being highest in the greens grown in soils which contained the most

phosphorus. The low-phosphorus soils gave more significant effects and more interactions than either the medium- or the high-phosphorus soils. There were three significant effects for fertilizer treatments—a positive effect for P, a negative effect for N, and a positive interaction effect for NP. Highly significant treatment × places interactions were given by the treatments NPKCa, NPK, NP, N, and P. In the medium-phosphorus soils N had a highly significant, and Ca a significant, positive effect. There were only two treatment × places interactions, one for NPKCa and one for N. The high-phosphorus soils gave a significant positive interaction effect for NP, and a significant negative interaction effect for NK. There was significant treatment × places interaction for N, but no significant effect, since in one experiment there was a significant negative effect and in the other a highly significant positive effect of about the same order.

In the 28 experiments the low-phosphorus soils included 8 additional experiments (experiments 2, 5, 7, 9, 13, 17, 29, 30), and the medium soils 1 additional experiment (experiment 24). There was also a group for trace-phosphorus soils (experiments 1 and 15). The results were

TABLE 16.—*Results of the experiments from the low-error group, showing the phosphorus content of turnip greens grown on low-, medium-, and high-phosphorus soils, the average effect of the fertilizer treatment, and the F values from the variance analysis of each group*

Fertilizer treatment	Low-P soils (14 experiments [1])		Medium-P soils (2 experiments [2])		High-P soils (2 experiments [3])	
	Average P content, 28 plots (dry basis)	Average effect of treatment, 224 plots	Average P content, 4 plots (dry basis)	Average effect of treatment, 32 plots	Average P content, 4 plots (dry basis)	Average effect of treatment, 32 plotes
	Percent		*Percent*		*Percent*	
NPKCa	0.5426	−0.0030††	0.7205	−0.0011††	0.6950	+0.0113
NPK	.5561	+.0024††	.6535	−.0040	.6925	−.0138
NPCa	.5496	−.0020	.7155	+.0061	.7375	+.0038
NP	.5550	+.0150**††	.7173	−.0025	.7650	+.0188*
NKCa	.4893	−.0034	.7098	+.0132	.6775	−.0031
NK	.4809	−.0018	.6870	−.0086	.7075	−.0256**
NCa	.4953	−.0011	.6783	−.0030	.7175	−.0056
N	.4913	−.0208**††	.6993	+.0806**††	.7050	+.0069††
PKCa	.5573	−.0001	.6398	+.0073	.7025	+.0069
PK	.5517	+.0002	.6033	−.0155	.7125	−.0056
PCa	.5529	−.0058†	.6338	+.0098	.6825	−.0056
P	.5648	+.0467**††	.6175	+.0106	.6900	+.0019
KCa	.5286	+.0024	.6260	+.0149	.7250	+.0000
K	.5215	−.0034	.6138	−.0013	.7075	−.0125
Ca	.5256	−.0005	.6140	+.0197*	.7125	−.0050
Check	.5241	------	.5883	------	.7100	------
Mean	.5304	------	.6573	------	.7088	------
Standard deviation	.0338	------	.0316	------	.0332	------
Odds at 19:1	.0178	.0063	.0455	.0161	.0478	.0169
Odds at 99:1	.0235	.0083	.0614	.0217	.0645	.0228
Treatment of F values:						
Residual as error	19.13**	------	8.49**	------	1.64	------
Place × treatment as error	8.25**	------	.10	------	.62	------
Place F values:						
Residual as error	204.95**	------	5.16*	------	7.82**	------
Place × treatment as error	88.38**	------	.60	------	2.97	------
Replication F value:						
Residual as error	7.29*	------	1.71	------	1.09	------

[1] Factorial experiment Nos. 3, 4, 10, 11, 14, 15, 16, 18, 19, 20, 21, 22, 23, 26.
[2] Factorial experiment Nos. 6, 27.
[3] Factorial experiment Nos. 12, 13.
*=Significant at odds of 19:1; **=significant at odds of 99:1; †=significant interaction of treatment × places at odds of 19:1; ††=significant interaction of treatment × places at odds of 99:1.

comparable to those for the low-error experiments except that the effects on low-phosphorus soils in the 28 experiments showed an even greater degree of significance.[7] Six treatments, P, Ca, N, NP, NK, and PK had a highly significant effect. P and Ca had a positive effect and N a negative effect. The interaction effect of NP was positive and that of NK and PK was negative. There was a highly significant treatment × places interaction for each of the 15 treatments.

In none of the other soil groups were there more than three significant effects or interactions of treatment and places. In the trace-phosphorus soils the treatments N and P had a highly significant positive effect and NP a significant positive interaction effect, and there was a significant interaction for N. In the medium-phosphorus soils N had a highly significant positive effect, whereas NK had a highly significant negative interaction effect. Significant treatment × places interaction was shown by NK and a highly significant interaction by N. The high-phosphorus group was identical with that in the low-error experiments.

In the analyses of variance for the low-error experiments (table 16), the *F* values for place on the basis of residual as error in the medium- and high-phosphorus soils were significant, but were very much smaller than the *F* values for place on the low-phosphorus soils. This was true for the 28 experiments [7] as well as for the 18 low-error experiments, but the differences in the *F* values were greater for the 28 experiments. The *F* values for treatment on the basis of residual as error were highly significant for the low- and medium-phosphorus soils of both the low-error and the 28 experiments, but were not significant for the high-phosphorus soils. When the *F* values for treatment were determined on the basis of interaction (place × treatments) as error they were highly significant for the low-phosphorus soils, but not significant for the medium- and high-phosphorus soils of the low-error experiments. The results were the same for the 28 experiments except that the *F* value for the medium-phosphorus soils showed signficance at the 5-percent level.

In order to determine the effect of the level of soil calcium upon the phosphorus content of the greens, the 24 low-phosphorus soils were grouped into low- and high-calcium soils and the effects of the fertilizer treatments studied. Since there were just 5 soils not included in the low-phosphorus group, only the latter could be studied for the effects of soil calcium. It was found that in both the low- and high-calcium soils the effects of the fertilizer treatments were those typical of the low-phosphorus soils in the low-error experiments shown in table 16. The effects for NP, N, and P were greater on the low-calcium soils than on the high-calcium soils. The treatment PCa had a significant negative interaction effect on the high-calcium soils, but not on the low-calcium soils.

In the analyses of variance there was a greater variation due to place on the high- than on the low-calcium soils. There was a greater variation due to treatment, however, on the low-calcium soils.

Effect of the Level of Organic Matter in the Soil

The effect of the level of organic matter on the phosphorus content of the greens was also determined. The soils were classified on the

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[7] Table not shown, but is available at the Mississippi Experiment Station.

basis of actual amount of organic matter present. In the low-error experiments there were only two groups of soils—the low group containing 0.48 to 1.20 percent organic matter, and the medium group containing 1.20 to 2.00 percent organic matter—since there was only one soil in the high organic-matter group.

TABLE 17.—*Results of the experiments from the low-error group, showing the average phosphorus content of turnip greens grown on soils containing different percentages of organic matter, the average effect of the fertilizer treatments, and the F values from the variance analysis of each group*

Fertilizer treatment	Soils containing 0.48 to 1.20 percent organic matter (6 experiments [1])		Soils containing 1.20 to 2.00 percent organic matter (11 experiments [2])	
	Average P content, 12 plots (dry basis),	Average effect of treatment, 96 plots	Average Ca content, 22 plots (dry basis)	Average effect of treatment, 176 plots
	Percent		*Percent*	
NPKCa	0.5645	−0.0118*††	0.5867	+0.0035
NPK	.5901	+.0152**†	.5827	−.0072*
NPCa	.5842	−.0029	.5924	+.0006
NP	.5695	+.0171**†	.6116	+.0106**†
NKCa	.5026	−.0070	.5504	+.0008
NK	.4922	−.0012	.5470	−.0078*††
NCa	.5185	−.0006	.5551	−.0032
N	.5281	−.0232**††	.5427	+.0038††
PKCa	.5789	−.0033	.5859	+.0046
PK	.5598	+.0020	.5846	−.0013
PCa	.5890	+.0000	.5700	−.0081*††
P	.6047	+.0495**††	.5750	+.0339**††
KCa	.5522	+.0019	.5641	+.0028
K	.5561	−.0115*	.5481	−.0009
Ca	.5449	−.0019	.5621	+.0034
Check	.5496	----------	.5480	----------
Mean	.5553	----------	.5691	----------
Standard deviation	.0336	----------	.0306	----------
Odds at 19:1	.0272	.0096	.0181	.0064
Odds at 99:1	.0362	.0128	.0246	.0087
Treatment *F* values:				
Residual as error	10.95**		9.54**	
Place × treatment as error	3.46**		2.29**	
Place *F* values:				
Residual as error	190.05**		464.22**	
Place × treatment as error	60.11**		111.26**	
Replication *F* value:				
Residual as error	14.43**		2.20*	

[1] Factorial experiment Nos. 10, 11, 15, 21, 23. 27.
[2] Factorial experiment Nos. 4, 6, 12, 13, 14, 16, 18, 19, 20, 22, 26.
*=Significant at odds of 19:1; **=significant at odds of 99:1; †=significant interaction of treatment × places at odds of 19:1; ††=significant interaction of treatment × places at odds of 99:1.

The results for the low-error experiments are given in table 17. There was a small and not significant increase in the mean phosphorus content of the greens with increased organic matter. In the total of 28 experiments there was a similar trend when the experiments were grouped so that the soil calcium and phosphorus were held at the low level.[7]

The effect of P was to significantly increase the phosphorus content of the greens, and NP had a significant positive interaction effect in the low-error experiments. The same effects were shown when the 28 experiments were grouped into low, medium, and high organic-matter

[7] Table not shown, but is available at the Mississippi Experiment Station.

soils.[7] When the phosphorus and calcium soil levels were held constant, the effect of applied phosphorus seemed to decrease with increased organic matter.

In the low organic-matter group of the low-error experiments, N produced a highly significant decrease in the phosphorus content of the greens, but no significant effect in the medium organic-matter group. This group, however, was made up of 11 experiments, 5 of which (experiments 4, 6, 13, 14, 19) gave a positive effect for nitrogen, highly significant when combined, and 6 of which (experiments 12, 16, 18, 20, 22, 26) gave a negative effect, also highly significant when combined. More of the nitrogen treatments had a significant effect in the low organic-matter group for both low-error and the 28 experiments than for the soil groups having a higher organic-matter content. Potassium showed a tendency to decrease the phosphorus content of the greens, but has a significant effect only in the low organic-matter group of the low-error experiments. The treatment NK gave a significant negative interaction effect in the medium organic-matter group of the low-error experiments, and a similar effect in the low organic-matter group of the total experiments.

All of the first-order treatments in the low-error experiments which give significant effect showed significant interactions for treatment $\times$ places except K in the low organic-matter group. In the 28 experiments there were significant interactions for N and P in all 3 organic-matter groups.

In the analyses of variance for the low-error experiments there was an increase in the F values for place when the organic matter increased. The variation due to treatment decreased, however, with an increase in organic matter.

Correlation Coefficients and Regression Equations

Correlation studies were made to bring out relations between soil properties and the phosphorus content of the greens. The plots showing significant places $\times$ treatment interaction and the mean for all plots were studied as in the case of calcium. The correlation coefficients and regression equations (table 18) show that the phos-

Table 18.—*Correlation coefficients and regression equations showing relations between exchangeable calcium and soil phosphorus, and the phosphorus in the greens for the mean and for plots showing significant places × treatment interaction*

| Plot | r | | R | Regression equation [1] | Standard error of estimate |
	Soil phosphorus	Exchangeable calcium	Soil phosphorus and exchangeable calcium		
NPKCa	0.2033	0.1942	0.2567	$0.5181 + 0.0082x + 0.0668x_1$	0.0965
NPK	.3022	.1739	.3237	$.4978 + .0132x + .0050x_1$	.0930
NP	.4987**	.1924	.5076*	$.4456 + .0247x + .0044x_1$	.0920
N	.6290**	.0827	.6306**	$.3191 + .0326x - .0023x_1$	.0916
P	.2430	.0831	.2455	$.5420 + .0091x + .0012x_1$	.0783
Mean	.5380**	.1258	.5590*	$.4399 + .0214x + .0007x_1$	.0690

[1] x = Soil phosphorus; x_1 = exchangeable calcium.

[7] Table not shown, but is available at the Mississippi Experiment Station.

* = Significant at odds of 19:1; ** = significant at odds of 99:1; † = significant interception of treatment $\times$ places at odds of 19,1; †† = significant interaction of treatment $\times$ places at odds of 99:1.

phorus in the greens from the N and NP plots as well as the mean phosphorus increased significantly as the soil phosphorus increased. The closest relation between soil phosphorus and that in the greens was shown by the data from the nitrogen plots. The multiple correlation coefficients for soil phosphorus and exchangeable calcium which were significant were only slightly greater than the simple coefficients for soil phosphorus.

Other soil properties as studied had less effect on the phosphorus in the greens than did exchangeable calcium. Simple correlation coefficients showed a negative effect of organic matter on the mean phosphorus and on that of the greens from the N and P plots, and a positive effect on the phosphorus in the greens from the NP, NPK, and NPKCa plots. Multiple coefficients were but slightly larger than the simple coefficients for soil phosphorus, suggesting that little or no information could be gained by the multiple studies.

Covariance Analyses

Multiple correlation coefficients indicated that soil phosphorus and exchangeable calcium were more closely related to the mean phosphorus in the greens than were soil phosphorus and organic matter or soil phosphorus and pH. Hence, covariance analyses were made to study the effects of these soil properties on the variation in the plant phosphorus attributed to place. The results of the analyses made for the greens grown on the check and NKCa plots are shown in table 19. The F value 9.00** for place on the check plots was reduced to 6.75** when the means were adjusted on the basis of soil phosphorus; to 8.67** when adjusted on the basis of pH; and to 5.67** when adjusted on the basis of soil phosphorus and exchangeable calcium. The soil phosphorus accounted for more of the variation due to place than did the other soil properties studied.

In a similar study of the NKCa plots, where normal growth was expected with no phosphorus added, the F value for place was 6.55**. This value was reduced to 3.91** when the means were adjusted on the basis of soil phosphorus; to 6.19** when adjusted on the basis of pH; and to 3.75** when adjusted on the basis of soil phosphorus and exchangeable calcium. Soil phosphorus accounted for approximately 40 percent of the variation attributed to place when plots received NKCa treatments, whereas it accounted for only 25 percent when plots were not fertilized. The greater variation within the check plots unaccounted for by soil phosphorus suggests that some of the variation due to place might have been the result of differences in growth between the NKCa and check plots.

A covariance analysis of the data from the N plots was also made, since the multiple coefficient (0.6306**) for soil phosphorus and exchangeable calcium (table 18) was greater than the corresponding coefficient determined for the NKCa plots. The adjusted F value for place was, however, still highly significant.

**Significant at odds of 99:1.

TABLE 19.—*Covariance analyses of the phosphorus content of turnip greens from the check and NKCa plots, showing the effects of soil phosphorus, hydrogen-ion concentration (pH), and exchangeable calcium in variation due to place*

Plot	Source of variation	Variance of P content of greens (Y)				Error of estimate based on soil phosphorus (X)				Error of estimate based on pH (X_1)				Error of estimate based on soil phosphorus and exchangeable calcium (X_2)				
		Degrees of freedom	SY^2	Mean square	F	Degrees of freedom	$SY^2-\frac{(SXY)^2}{SX^2}$	Mean square	F	Degrees of freedom	$SY^2-\frac{(SX_1Y)^2}{SX_1^2}$	Mean square	F	Degrees of freedom	R^2	$(1-R^2)SY^2$	Mean square	F
Check	Total	55	0.618447			54	0.495244			54	0.617622			53	0.2883	0.440115		
	Place	27	.554546	0.020539	9.00**													
	Error	28	.063901	.002282		27	.063901	0.002367		27	.063901	0.002367		26	0	.063901	0.002458	
	Difference for testing adjusted means	27				27	.431343	.015976	6.75**	27	.553721	.020508	8.67**	27		.376214	.013933	5.67**
NKCa	Total	55	.708763			54	.474563			54	.695803			53	.3568	.455876		
	Place	27	.61056	.022613	6.55**													
	Error	28	.096707	.003454		27	.096707	.003582		27	.096707	.003582		26	0	.096707	.003720	
	Difference for testing adjusted means	27				27	.377856	.013995	3.91**	27	.599096	.021189	6.19**	27		.359169	.013303	3.57**

**Significant at odds of 99:1.

EFFECT OF RAINFALL AND IRRIGATION

As stated previously, records were secured of the rainfall during the growing period of each crop. The relations between the total rainfall and the average calcium and phosphorus content of the greens grown in the same place but in different seasons, or in different years, or both, were studied. The results, presented in table 20, do not show any consistent effect of rainfall on the average calcium and phosphorus content of the greens either for season or for year.

Correlation coefficients were employed to study the relation between the calcium and phosphorus in the greens and rainfall on the check plots. The coefficient for calcium (0.1527) as well as that for phosphorus (0.0361) indicated that rainfall was a minor factor in the variation due to place.

TABLE 20.—*Effect of rainfall in different seasons, at the same place, and the effect of irrigation on the calcium and phosphorus content of turnip greens*

Location	Spring, 1939			Fall, 1939			Spring, 1940			Fall, 1940		
	Rainfall	Average Ca content	Average P content	Rainfall	Average Ca content	Average P content	Rainfall	Average Ca content	Average P content	Rainfall	Average Ca content	Average P content
	Inches	*Per-cent*	*Per-cent*	*Inches*	*Per-cent*	*Per-cent*	*Inches*	*Per-cent*	*Per-cent*	*Inches*	*Per-cent*	*Per-cent*
Blairsville, Ga	10.32	3.17	0.601				5.46	3.03	0.549			
Experiment, Ga	11.11	2.47	.565							4.67	2.82	0.440
Crystal Springs, Miss				5.67	2.00	0.666	14.28	2.13	.520			
Poplarville, Miss	4.42	2.40	.492	4.42	2.12	.419	11.38	1.99	.557			
Stoneville, Miss				4.12	2.83	.720	9.46	2.88	.697			
Perkins, Okla	5.59	2.67	.516				5.41	2.97	.487			
Woodward, Okla	5.87	3.17	.463				2.59	3.24	.549			
Do [1]							3.23	3.46	.606			
Winter Haven, Tex. [1]				2.94	2.34	.658				2.00	2.21	.648
Norfolk, Va				9.56	2.29	.516				10.77	2.71	.515

[1] Crops were also irrigated.

The effect of rainfall on the NPKCa plots was also studied. These plots were selected because it seemed likely that the effects of rainfall on the calcium content of the greens might be more apparent in the plots having a complete fertilizer. The F value for the variation due to place was 16.61**. This value was reduced to 15.86** when the means were adjusted for total rainfall. When the phosphorus content of the greens was similarly studied, the F value for the variation due to place was 8.87**. Adjusting the means for total rainfall reduced the F value to 8.14**. Thus rainfall seems to account for only a small part of the variation in the calcium and phosphorus content of the greens due to place.

Irrigation increased the calcium and phosphorus content of turnip greens grown the same season at the same place (Woodward) on irrigated and nonirrigated plots. The F value for irrigation in an analysis of variance of the calcium data was 15.96** and in a similar analysis of the phosphorus data, 50.50**.

YIELD

Yield records were taken in 12 of the 30 experiments and for 1 crop at Heavener, Okla., which was not analyzed for minerals.

Eleven of the crops were grown in 1940. The yields for these experiments, their means, and the standard deviation are given in table 21. The average yield ranged from 61.74 gm. per foot of row at Experiment, Ga., to 358.46 gm. at Perkins, Okla. Significant treatment effects are listed in table 22. The application of nitrogen produced a highly significant increase in yield in 8 of the 13 experiments. At one place only (Woodward) was there a significant decrease due to nitrogen and this was on the nonirrigated plots. The yield at Blairsville was significantly increased by P and K and the interaction effects of NP and NK were positive. Potassium also significantly increased the yield at Norfolk. Significant positive interaction effects were given by PCa and PKCa at Woodward and by NPKCa at College Station.

The average effects of treatment on yields for the 13 experiments and the 3 error groups, and the F values for the analyses of variance are given in table 23. Nitrogen had the greatest effect of any of the fertilizer treatments. It produced an average increase in yield of 72.71 gm. per foot of row. There was an interaction of N $\times$ places in all groups. Potassium significantly increased the yield in the combined analysis, and in the low- and medium-error groups. The interaction of K $\times$ places was significant in the low-error group. Phosphorus produced a significant increase in yield in the low-error group and gave a significant treatment $\times$ places interaction in the medium-error group. Calcium had a significant positive effect in the low-error group. There were significant treatment $\times$ places interactions for NP and NK in the low-error group, and for PCa and P in the medium-error group. The F values from the analyses of variance show that place accounted for much more of the variation than did treatment, although the latter was highly significant.

As it seemed possible that the negative effect of N on the Ca and P in the greens might have been due to the increased yield resulting from the N treatment, a covariance analysis was made adjusting the mean calcium and phosphorus in the greens on the basis of yields (table 24). The calcium and phosphorus data for Iowa Park and Heavener were eliminated. It was also necessary to eliminate from the phosphorus study the data from experiments in which N significantly increased the phosphorus content of the greens. Results of the covariance analyses shown in table 24 indicate that in these experiments the difference in the calcium and phosphorus content of greens grown on nitrogen plots and those grown on plots receiving no fertilizer could be accounted for by increased yield. Attention, however, should be called to the fact that increased yields are not necessarily accompanied by a decrease in calcium and phosphorous in the greens, especially the latter. Thus, at Stoneville, the addition of nitrogen produced a large increase in yield as well as a significant increase in the phosphorous of the greens, while at Norfolk an increase in yield occurred with no decrease in either calcium or phosphorus in the plant. Differences in yield also failed to account for the significant variation in the calcium and phosphorus content of the greens from the experiments where yield records were taken.

TABLE 21.— *Average yield of turnip greens grown by a factorial design of 16 duplicate fertilizer treatments in 13 experiments*

Experiment No.	Location	Year	Average yield per foot of row from duplicate plots fertilized with—									
			NPKCa	NPK	NPCa	NP	NKCa	NK	NCa	N	PKCa	PK
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
2	Blairsville, Ga.	1940	184.20	158.75	84.35	107.05	120.65	95.25	70.75	52.65	66.20	45.40
4	Experiment, Ga.	1940[1]	72.60	60.80	47.20	76.20	70.05	57.15	66.20	53.50	62.60	58.05
7	Crystal Springs, Miss.	1940	308.35	213.35	233.35	146.65	255.00	226.65	231.65	200.00	30.35	19.75
11	Poplarville, Miss.	1940	276.00	288.00	220.15	236.00	224.70	282.80	223.15	243.80	59.00	43.80
13	Stoneville, Miss.	1940	449.15	476.65	401.65	389.00	524.00	370.00	425.00	467.50	154.35	148.20
17	Perkins, Okla.	1940	373.75	408.25	333.80	343.35	357.45	314.80	355.70	377.35	401.00	374.65
19	Woodward, Okla.	1940	111.55	105.25	117.00	124.25	115.20	91.65	151.50	117.05	156.95	142.45
20	do[2]	1940	311.15	191.45	206.85	146.05	140.60	305.70	257.05	186.85	129.75	112.45
15-A[3]	Heavener, Okla.	1940	157.85	129.70	114.30	82.55	89.80	83.25	98.90	69.85	36.30	62.40
24	College Station, Tex.	1940[1]	245.20	270.75	205.55	279.20	206.95	244.75	222.50	208.40	249.45	289.20
25	Iowa Park, Tex.	1939[1]	154.20	280.95	270.75	267.30	231.70	220.05	310.75	227.60	221.80	236.75
27	Winter Haven, Tex.	1940[1]	234.25	204.65	184.15	205.25	232.55	282.55	247.95	239.50	193.30	213.65
30	Norfolk, Va.	1940[1]	180.50	151.00	163.50	130.00	145.50	150.00	131.50	133.50	90.00	81.50

Experiment No.	Location	Year	Average yield per foot of row from duplicate plots fertilized with—Continued						Mean	Standard Deviation	Odds of—	
			PCa	P	KCa	K	Ca	Check			19:1	99:1
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Percent*			
2	Blairsville, Ga.	1940	47.20	40.85	43.55	39.00	36.30	46.30	77.40	20.87	44.46	63.24
4	Experiment, Ga.	1940[1]	59.90	56.95	58.05	52.65	67.00	68.95	61.74	18.02	38.38	53.09
7	Crystal Springs, Miss.	1940	21.65	28.35	30.00	22.35	34.85	10.00	125.76	65.77	140.15	193.83
11	Poplarville, Miss.	1940	52.80	68.80	58.15	95.30	34.80	93.00	156.63	37.14	79.14	109.43
13	Stoneville, Miss.	1940	169.20	177.30	145.65	197.50	134.80	156.65	299.16	79.56	169.53	234.47
17	Perkins, Okla.	1940	319.35	366.50	380.15	348.35	352.00	323.85	358.46	55.55	118.37	163.71
19	Woodward, Okla.	1940	156.95	137.90	148.80	127.90	123.40	112.45	127.52	25.50	54.33	75.15
20	do[2]	1940	142.40	130.65	97.10	146.00	88.90	124.30	169.87	46.34	98.74	136.58
15-A[3]	Heavener, Okla.	1940	42.65	27.25	60.80	38.10	34.45	31.75	72.49	27.79	59.23	81.88
24	College Station, Tex.	1940[1]	270.75	177.20	225.40	185.65	198.50	296.25	235.98	44.45	94.72	130.98
25	Iowa Park, Tex.	1939[1]	272.45	226.40	283.65	354.65	288.35	377.10	264.03	70.97	151.23	209.13
27	Winter Haven, Tex.	1940[1]	172.50	169.05	230.35	149.60	218.05	162.85	208.14	35.19	74.98	103.72
30	Norfolk, Va.	1940[1]	64.50	62.50	70.00	87.50	88.00	60.50	111.88	17.50	37.31	51.56

[1] Fall crops. Undesignated crops were grown in the spring.   [2] Plots were irrigated.   [3] No mineral analyses were made on the 1940 crop at Heavener.

TABLE 22.—*Significant effects of the 16 fertilizer treatments on the yield of turnip greens from 12 experiments*

Experiment No.	Location	Average effect of indicated fertilizer treatment on yield per foot of row															Odds of—	
		NPKCa	NPK	NPCa	NP	NKCa	NK	NCa	N	PKCa	PK	PCa	P	KCa	K	Ca	19:1	99:1
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.		
2	Blairsville, Ga.				+20.07*		+27.57**		+63.61**				+28.69**		+33.44**		15.72	22.36
4	Experiment, Ga.																13.57	18.77
7	Crystal Springs, Miss.								+202.29**								49.55	68.53
11	Poplarville, Miss.								+186.64**								27.98	38.69
13	Stoneville, Miss.								+277.41**								59.94	82.90
17	Perkins, Okla.																41.85	57.88
19	Woodward, Okla.								−21.67*								19.21	26.57
20	do.								+96.94**	+39.23*							34.91	48.29
15-A	Heavener, Okla.	+46.35**							+61.56**			+48.52**					20.94	28.95
24	College Station, Tex.																33.49	46.31
25	Iowa Park, Tex.																33.47	73.94
27	Winter Haven, Tex.								+41.15**								26.51	36.67
30	Norfolk, Va.								+72.63**						+15.25*		13.19	18.23

*= Significant at odds of 19 : 1; **=significant at odds of 99 : 1.

The regression equation using mean percentage of calcium as a dependent factor (Y) on exchangeable calcium (X) and yield (X_1) is $Y = 2.093 + 0.1208X + 0.0005X_1$. The multiple correlation coefficient, $R = 0.7292*$, is but slightly larger than the simple correlation coefficient, $r = 0.7138**$, for exchangeable calcium. The regression equation using average percentage of calcium from the N plots as a dependent factor on the same independent factors is $Y = 2.2396 + 0.1027X - 0.0007X$. For these plots $R = 0.6069$ where $r_{xy} = 0.5816$ and $r_{x1}y = -0.2830$.

Regression equations using percentage of phosphorus as a dependent factor were not studied because the variation in the effect of N on percentage of phosphorus limited homogeneous data to a few experiments.

TABLE 23.—*Results of the total experiments and of the error groups, showing the average yield of turnip greens per foot of row, the average effects of the fertilizer treatments on yield per foot of row, and the F values from the variance analysis of each group*

Fertilizer treatment	Total group (13 experiments [1])		Low-error group (5 experiments [2])		Medium-error group (5 experiments [3])		High-error group (3 experiments [4])	
	Average yield, 26 plots	Average effect of treatment, 208 plots	Average yield, 10 plots	Average effect of treatment, 80 plots	Average yield, 10 plots	Average effect of treatment, 80 plots	Average yield, 6 plots	Average effect of treatment, 48 plots
	Gm	Gm	Gm	Gm	Gm	Gm	Gm	Gm
NPKCa	235.29	+5.36	141.34	+3.84	288.07	+17.2)*†	303.90	−11.85
NPK	226.12	+6.73	121.10	+3.62	272.62	+5.74 †	323.65	+13.58
NPCa	198.66	−3.56	105.27	−1.29	230.10	+3.46	301.92	−19.06
NP	195.22	+3.98	104.01	+7.29†	242.97	−2.72	267.65	+9.63
NKCa	208.79	−1.82	108.24	+2.08	232.47	−7.27	336.90	+.75
NK	209.58	+5.79	95.46	+4.41††	286.12	+5.86	272.23	+7.98
NCa	214.86	+3.72	103.77	+3.26	261.39	−5.53	322.47	+19.88
N	198.60	+72.71**††	85.31	+35.71**††	252.02	+64.85**††	298.37	+147.49**††
PKCa	142.39	+.34	82.41	+2.32	206.50	+5.64	135.50	−11.80
PK	140.59	+8.92	77.96	+6.02	206.75	+14.87	134.70	+3.85
PCa	137.87	+2.85	74.24	−1.15	191.56	+8.26	154.43	+.50
P	127.67	+1.89	65.09	+12.44*ꞌ	180.44	+3.16††	144.02	−17.84
KCa	140.89	−1.01	76.24	+1.25	198.23	−1.40	153.08	−4.11
K	141.89	+12.32*	69.03	+12.53**††	184.98	+17.35*	191.50	+3.59
Ca	130.72	+3.30	69.83	+9.92*	178.45	−4.90	152.67	+5.94
Check	143.38	....	63.99	....	200.05	....	181.25	....
Mean	174.53	....	90.21	....	225.80	....	229.64	....
Standard deviation	46.40	....	22.21	....	44.33	....	72.33	....
Odds at 19:1	27.44	9.70	21.16	7.48	42.26	14.94	88.98	31.46
Odds at 99:1	37.93	13.41	29.27	10.35	58.43	20.66	123.06	43.51
Treatment F values:								
Residual as error	18.34**	....	9.99**	....	7.23**	....	7.20**	....
Place × treatment as error	5.80**	....	.79	....	2.58**	....	2.09ꞌ	....
Place F values:								
Residual as error	128.46**	....	51.07ꞌ*	....	105.58**	....	51.42**	....
Place × treatment as error	40.63**	....	4.04**	....	37.61**	....	14.92**	....
Replication F value:								
Residual as error	1.75	....	1.87	....	2.80*	....	1.08	....

[1] Factorial experiment Nos.: 2, 4, 7, 11, 13, 17, 19, 20, 15-A, 24, 25, 27, 30.
[2] Factorial experiment Nos.: 2, 4, 15-A, 19, 30.
[3] Factorial experiment Nos.: 11, 17, 20, 24, 27.
[4] Factorial experiment Nos.: 7, 13, 25.

*=Significant at odds of 19:1; ** = significant at odds of 99:1; †=significant interaction of treatment × places at odds of 19:1; ††=significant interaction of treatment × places at odds of 99:1.

TABLE 24.—*Covariance analyses indicating the relationship of yield to the calcium and phosphorus content of turnip greens*

Source of variation	Calcium[1]								Phosphorus[2]							
	Variance of calcium content of greens (y)				Error of estimate based on average yields (x)				Variance of phosphorus content of greens (y)				Error of estimate based on average yields (x)			
	Degrees of freedom	Sy^2	Mean square	F	Degrees of freedom	$Sy^2 - \frac{S(xy)^2}{Sx^2}$	Mean square	F	Degrees of freedom	Sy^2	Mean square	F	Degrees of freedom	$Sy^2 - \frac{S(xy)^2}{Sx^2}$	Mean square	F
Total	21	88.6209							15	0.9017						
Place	10	69.8770							7	.6049						
N-level	1	11.3868	11.3868	**15.48					1	.1532	0.1532	*7.47				
Error	10	7.3571	0.7357		9	2.9138	0.3238		7	.1436	.0205		6	0.0719	0.0120	
N+error	11	18.7439			10	4.1482			8	.2968			7	.0770		
Difference for adjusting means					1	1.2344	1.2344	3.81					1	.0051	.0051	(3)

[1] Experiments used in calcium analysis: 2, 4, 7, 11, 13, 17, 19, 20, 24, 27, 30.
[2] Experiments used in phosphorus analysis: 2, 7, 11, 17, 19, 20, 27, 30.
[3] Not significant.
° = Significant at odds of 19:1; ** = significant at odds of 99:1; † = significant interaction of treatment × places at odds of 19:1; †† = significant interaction of treatment × places at odds of 99:1.

DISCUSSION

The results from the data which have been presented in this study show the effects of the same fertilizer treatments on the calcium and phosphorus content of turnip greens when they were grown under a variety of environmental conditions. It is to be expected, therefore, that the effects of the fertilizer treatments would vary with the place where the greens were grown. In some respects, however, there was a rather striking similarity between places in the effects of certain treatments, notably those for nitrogen, calcium, and phosphorus. Nitrogen significantly decreased the calcium content of the greens in 24, and the phosphorus content in 12, of the experiments. This is in accord with the effect of nitrogen fertilizers on pasture grasses reported by several investigators and reviewed by Vandecaveye (26). Other investigators (28, 9), have noted the depressing effect of applied nitrogen on the calcium content of plants, but have reported an increase in the phosphorus content. Since there were 6 experiments in which the phosphorus was significantly increased by nitrogen fertilizer, it would seem that its effect on phosphorus was more dependent upon factors associated with place, including other soil factors, than was its effect on calcium.

The increase in the calcium content of the greens from applied calcium confirms the results of previous studies on vegetables (18, 21), and other plants (17, 20). The greatest responses to added calcium were observed when the greens were grown on soils containing the east calcium. When calcium was added to the soil it also showed a tendency to increase the phosphorus content of the greens, although the results were not consistent. Varying effects of applied calcium on the phosphorus content of plants have been reported by Ames and Schollenberger (2) and by Beeson (6), the results obtained depending upon the soil in which the plants were grown.

The effect of phosphorus on the phosphorus content of the greens was almost as great as that for calcium on the calcium content of the greens. Eighteen of the 27 experiments showing an increase in phosphorus gave significant increases. The soils in all of the 18 experiments were low in phosphorus. There have been many reports concerning the effect of phosphorus fertilizers on the phosphorus in plants which would seem to agree with these findings. Of particular interest are those of Bishop (7), Brown (8), and Wimer (29). In the majority of the experiments, the combination of phosphorus and nitrogen produced a positive interaction effect on the phosphorus in the greens, the effect being highly significant in the total experiments and in the low-error group. In this connection it is interesting to note that the interaction effect of NP was greatest on the low organic-matter soils (table 17).

The effect of applied phosphorus on the calcium content of the greens like that of calcium on the phosphorus content of the greens, seemed to depend upon the soil in which the greens were grown. The tendency of phosphorus was to decrease the calcium, there being a significant negative effect of three places and in the total experiments. A significant increase in phosphorus occurred, however, at three places.

The effect of place is the most striking result brought out by the analysis of the data on the calcium and phosphorus content of the greens. The variation in the average calcium and phosphorus in the

greens associated with place is graphically presented in figures 2 and 3, respectively. In the low-error groups of the variance analyses the effect of place was 13 times greater for calcium and 20 times greater for phosphorus than was the effect of treatment, although the latter had a highly significant effect. The much greater influence of place when compared to fertilizer treatment, on the calcium and phosphorus content of clover, has been reported by Myers and Metzger (*16*).
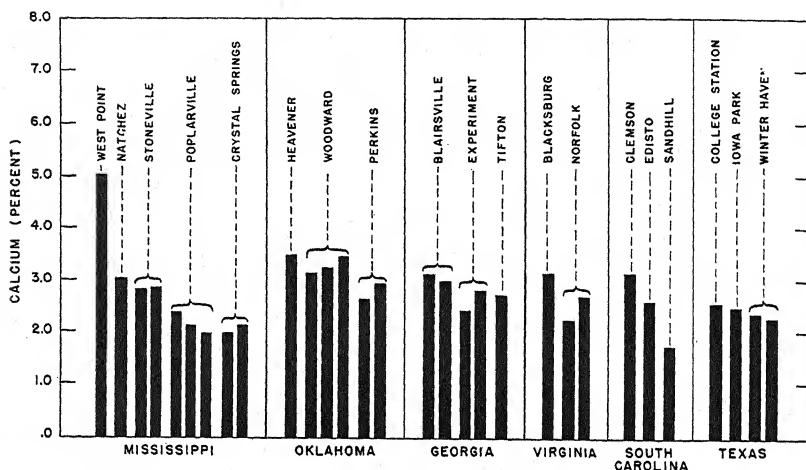
FIG. 2.—Average calcium content in each of 30 crops of turnip greens.
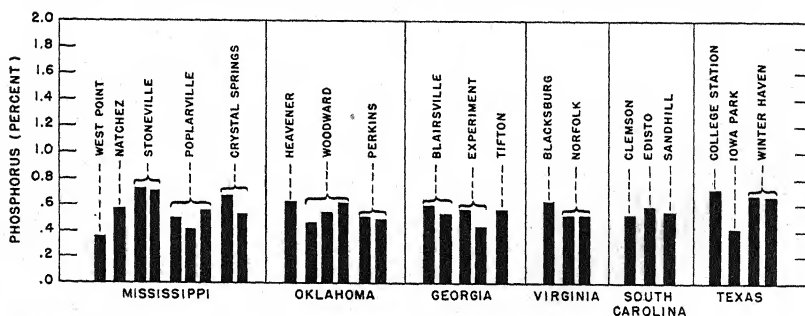
FIG. 3.—Average phosphorus content in each of 30 crops of turnip greens.

In a study of the composition of bluegrass in different parts of Ohio, Forbes, Whittier, and Collision (*12*) found that, "While the mineral content of vegetable crops is without doubt the resultant of a considerable number of varying factors, the most important of these is the composition of the soil." There can be no doubt that the composition of the soil is one of the most important factors affecting the mineral content of vegetables, and it would have been desirable to make much more complete investigations of this relationship at each place than was done. Such complicated studies were not feasible, but some of the soil properties which were studied indicated that soil had a more marked effect than did fertilizer treatments. When the soils were classified according to the amount of calcium or phosphorus

present and the effects of the fertilizer treatments determined for the different classes, it was found that soil calcium had a greater effect on the calcium content of the greens, and soil phosphorus on the phosphorus content of the greens, than did the respective minerals applied as fertilizer (tables 8 and 16). The greens grown on the high-calcium soils contained 33 percent more calcium on an average than those grown on the low-calcium soils. The greatest effect for applied calcium was shown by the trace-calcium soils, the increase being 0.135 percent. The average phosphorus content of the greens from the high-phosphorus soils was about 34 percent greater than that from the low-phosphorus soils. Only the low-phosphorus soils gave any significant effect for applied phosphorus, the increase in the phosphorus content of the greens being 0.0467 percent. In this connection, however, attention should be called to the differences in the soil levels of calcium and phosphorus as compared with the amount added as fertilizer. Differences between the calcium content of the trace- and high-calcium soils were about 2,000 pounds per acre, whereas only 120 pounds of calcium per acre was added as fertilizer. The difference between the low- and high-phosphorus soils was about 300 pounds per acre and only 60 pounds of P_2O_5 was added as fertilizer.

In the study of the relationships between soil properties and the calcium content of the greens the positive relationship between exchangeable calcium and plant calcium was the most significant. The experiments at West Point furnish a striking illustration of this observation. The West Point greens contained over 44 percent more calcium than those from any other place, and the amount of exchangeable calcium in the soil was much greater than that of any other place. The correlation coefficients for exchangeable calcium of the soil and the phosphorus in the greens were positive but not significant (table 18). The greens, however, which were grown in soil with an extremely high exchangeable calcium content had a low phosphorus content.

Although the rapid test for measuring available soil phosphorus is not as accurate as the test for measuring exchangeable calcium in the soil, the correlation coefficient showed a significant relationship between the available soil phosphorus level and the phosphorus content of the greens. The closest relationship between soil and plant phosphorus was given by the data from the nitrogen plots. This suggests that insufficient nitrogen in the soil was the limiting factor in the utilization of soil phosphorus by the plant.

Exchangeable calcium and hydrogen-ion concentration are so closely related that similar trends in effects on the mineral composition of the greens were to be expected. Both the correlation and the covariance analyses showed that exchangeable calcium has a greater effect than hydrogen-ion concentration on the calcium and phosphorus content of the greens.

Outstanding effects for organic matter were not demonstrated by the analysis of the data obtained in these investigations, although some interesting results were observed. The average calcium and phosphorus content of the greens increased with increased organic matter. The variation in each of the two minerals due to place was considerably less in the high organic-matter soils than it was for those lower in organic matter (tables 9 and 17).

A study of the various effects of the fertilizer treatments in the experiments which were repeated in successive seasons at the same location indicates that weather conditions may have had as great an influence as soil on the mineral composition of the plant. Thus the experiment at Norfolk in the fall of 1939 showed no effect on the phosphorus in the greens for any treatment, but the experiment for the fall of 1940 at the same place showed significant effects for four treatments (table 14). Even greater differences in the effects of the fertilizer treatments on calcium were found between the fall 1939 and the spring 1940 experiments at Stoneville. There were no significant effects for the fall experiment, but there were six significant effects for the spring experiments (table 6).

From an analysis of the data, rainfall did not show any consistent effect on the calcium and phosphorus content of turnip greens. The many other factors related to place which caused variations in the mineral content of the greens masked the effects which rainfall may have had. When, however, the greens were grown at the same place, during the same season, thereby eliminating variations due to place and season, it was found that irrigation significantly increased the calcium and phosphorus content of turnip greens. This is in agreement with the findings of Daniel and Harper (*10*) for phosphorus but not for calcium. They found that the phosphorus in hay increased with high rainfall but that the calcium decreased.

Both place and fertilizer treatments had an important effect on yield, but the effect of place was much greater than that of fertilizer treatments. It seems probable that the principal factors related to place which caused variations in yield were soil fertility and rainfall, but these factors could not be adequately studied because the data were insufficient.

It was to be expected that the application of nitrogen would increase the yield of turnip greens, as the majority of southern soils, including those on which the greens were grown, are rather low in nitrogen. The covariance analyses indicate that the decrease in the percentage of calcium and phosphorus in the greens resulting from the use of nitrogen fertilizer is associated with the increase in the amount of plant tissue (table 24). The results at Stoneville and Norfolk, however, demonstrate that an increase in yield is not always accompanied by a decrease in the calcium and phosphorus content of the plant. It is probable that results comparable to these would have been secured in other experiments had complete yield data been available for analysis. It should be noted also that while nitrogen decreased the calcium and phosphorus in the greens, increased yields resuled in an increase in the amount of plant calcium and phosphorus produced per foot of row.

The data on yield and on the calcium and phosphorus content of the greens (tables 5, 13, and 21) show that the variation due to place would still be significant when the effect of yield on the calcium and phosphorus content of the greens was eliminated. Although the data on yield are incomplete, the *F* values from the covariance analyses of the calcium and phosphorus in the greens grown on the check, NPK, and KNCa plots support the foregoing statement (tables 12 and 19).

SUMMARY AND CONCLUSIONS

Nineteen nonfactorial experiments were conducted at 12 localities, and 30 factorial experiments were conducted at 19 localities to determine the effects of place and of fertilizer treatments on the calcium and phosphorus content of turnip greens. Certain soil factors and rainfall were also studied in the factorial experiments. The plan for the nonfactorial experiments was to use a uniform fertilizer treatment at each place, the treatment depending upon the best soil practices in that locality. There were 10 replicates in each experiment. For the factorial experiments a $2 \times 2 \times 2 \times 2$ design was used for applications of N, P, K, and Ca in all possible combinations at a high and a low level for each nutrient. The same amount of each fertilizer was used in all experiments.

Seed of the variety Seven Top from the same source, as well as uniform methods of planting and fertilizing, were used. Soil samples were taken from 29 of the sites where the turnip greens were grown and were analyzed for calcium, total exchange capacity, exchangeable calcium, phosphorus, magnesium, organic matter, and pH values.

Samples of turnip greens harvested at a good marketable stage were prepared for analysis by washing in tap and distilled water, drying, and grinding in a porcelain ball mill. The whole plant was used with the exception of damaged leaves, a small portion of the stems next to the root, and the root. Methods of the Association of Official Agricultural Chemists were employed for the determination of calcium and phosphorus.

The results of the nonfactorial and of the factorial experiments were analyzed statistically. The analysis of variance was applied to the data from the factorial experiments. The method of Yate was used to determine the effect of each fertilizer treatment.

In both the nonfactorial and factorial experiments, there was a marked variation in the calcium and phosphorus content of the greens grown at different places. Significant differences in both elements were also observed in vegetables grown at the same place but in different seasons.

Results from the factorial experiments showed that conditions associated with place caused from 13 to 20 times more variation in the calcium and phosphorus content of greens than did the fertilizer in the amounts used, and that the effects produced by the different treatments varied with the season.

The soil properties total calcium, total phosphorus, exchangeable calcium, hydrogen-ion concentration, and organic matter were studied. Covariance and correlation analyses showed that these properties of the soil accounted for an important part, but not all, of the variation in the mineral content that was attributed to place. Results noted were as follows:

(1) The calcium content of the greens increased significantly as exchangeable calcium, pH, and the level of soil calcium increased.

(2) Exchangeable calcium did not have a significant effect on the phosphorus content of the greens.

(3) There was a small but not significant increase in both the calcium and phosphorus content of greens as the organic matter of the soil increased.

(4) The phosphorus content of greens grown on high-phosphorus soils was significantly greater than that of greens grown on low-phosphorus soils.

The data on rainfall showed no consistent relationship between the total amount of rainfall and the calcium and phosphorus content of the greens. At Woodward, however, both the plant calcium and phosphorus were significantly increased by irrigation.

The effects of fertilizer treatments, though not as great as that of place, were highly significant. The following effects were noted:

Nitrogen produced the most marked effect on the mineral content of the greens. It significantly decreased the calcium content of the greens in 24 of the 30 experiments. The average depression of calcium by nitrogen was approximately 0.36 percent. The effect of nitrogen on the phosphorus content of greens varied with season as well as with place. In general, the effect was a negative one, although in 6 experiments the phosphorus was significantly increased. There was a highly significant interaction for the effect of nitrogen on both the calcium and the phosphorus content of the greens, emphasizing the fact that the effects produced by nitrogen on different soils were not uniform.

Calcium applied as gypsum increased calcium in the greens on an average of 0.06 percent. The effect produced by Ca, however, varied greatly in the different experiments. Calcium showed a tendency to increase the phosphorus content of the greens, but the increase was significant in only 3 experiments and was not significant for the average of 29 experiments.

Applied phosphorus decreased the average plant calcium 0.046 percent, but it increased the average plant phosphorus 0.0537 percent. This was the greatest effect produced by any treatment on the phosphorus content of the greens. Both effects were highly significant and were characterized by significant interactions of treatment $\times$ places.

Potassium had no marked effect on either the calcium or the phosphorus. There were a few experiments, however, in which significant effects were produced.

The NK treatments had a significant negative interaction effect on both calcium and phosphorus. The effect on calcium was more pronounced than the effect on phosphorus.

The NP treatments had a significant positive interaction effect on phosphorus, but a significant negative effect on calcium. The variation in this effect caused by place was greater for phosphorus than for calcium.

Soils were classified according to the amount of calcium, phosphorus, and organic matter present, and the effects of the fertilizer treatments in each classification were determined. Treatments, other than N and P, produced greater effects on plant calcium when soils were low in calcium. The typical effects of N and of P, and the interaction effects of NP on phosphorus, were also more intense on low phosphorus-low calcium soils than on low phosphorus-high calcium soils. Applied P had no effect on the phosphorus of the greens grown on high-phosphorus soils.

Yield records were obtained for 13 experiments. The average yield was 174.53 gm. per foot of row. The significance of both main effects and interaction effects varied with the place. The low-error experi-

ments showed that yield was increased significantly by the applications of N, P, K, and Ca, but the greatest increase was produced by N. This average increase was approximately one-third of the total yield. The variation in yield caused by place was about seven times greater than that caused by applications of fertilizer treatments.

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CHEMICAL COMPOSITION OF CERTAIN FORAGE CROPS AS AFFECTED BY FERTILIZERS AND SOIL TYPES [1]

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INTRODUCTION

The quality of forage crops as livestock feeds depends upon many factors, including the kind of forage, its stage of maturity, its palatability, and its chemical composition. Although it has long been recognized that forage crops produced on certain soils make better quality livestock feed than the same crops produced on other soils under different or similar climatic conditions, the knowledge that certain factors of feeding quality are dependent upon the content of mineral constituents as well as upon the protein and other organic constituents of the crops is of relatively recent origin. The comprehensive review of Vandecaveye (4)[3] and Beeson (2) pertaining to investigations of the chemical composition of crops reveals that the composition of forage crops and, therefore, their feeding quality are affected by applications of fertilizers and by differences in soil properties as manifested by soil types.

In 1932 the authors (5) reported the results of 265 field-plot experiments designed to study the effect of different fertilizer treatments on the yield of crops produced on the major agricultural soil types of the State of Washington. Samples of many of the forage crops in these and subsequent experiments were saved for chemical analyses. The chief object of the present paper is to present the analytical results, and also a general interpretation of the effects of fertilizer treatments and soil types on the nitrogen, phosphorus, potassium, and calcium content of mixed pasture grasses, mixed hay, alfalfa hay, and oats and wheat cut for hay at the milk stage of maturity.

EXPERIMENTAL PLAN

FERTILIZER TREATMENTS

The samples of crop material used for chemical analysis were obtained from two series of fertilizer experiments carried on over a period of years on different soil types located largely in western Washington. The fertilizer treatments used in the two series of experiments are shown in table 1. The first series was designed for studies of pasture grass exclusively, and samples of herbage were obtained from eight of the plots. The part of each plot from which the samples of herbage were

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taken was enclosed by a fence and the remaining part was accessible for pasturing. The fertilizer treatments for the other series served for the study of forage crops other than pasture herbage.

The fertilizers were broadcast in the spring in all cases. For wheat, oats, and new seedings of alfalfa the fertilizer material was mixed with the soil by disking or harrowing before the crops were planted. If more than one annual application of fertilizer was made before crop samples were taken for chemical analysis, this is indicated in the table giving the analytical results. All the experiments were designated by a number preceded by a capital letter in accordance with the system used previously (*5*). The letter preceding each number indicates the year during which the experiment was in progress and the crops were sampled. Thus, B represents the year 1929; C, 1930; D, 1931; E, 1932; and F, 1933. The following symbols are used: N, nitrogen (N); P, phosphoric acid (P_2O_5); K, potash (K_2O); and Ca, lime hydrate or carbonate.

TABLE 1.—*Fertilizer treatments in the 2 series of experimental plots from which crop materials used for chemical analysis were obtained*

EXPERIMENTAL SERIES 1

Plot [1]	Fertilizer material	Quantity applied per acre
		Pounds
Check	None	None
P	16 percent superphosphate	600
PK	16 percent superphosphate	600
	50 percent muriate of potash	200
PKCa	16 percent superphosphate	600
	50 percent muriate of potash	200
	Hydrated lime	1,000
NPKCa	20 percent sulfate of ammonia	250
	16 percent superphosphate	600
	50 percent muriate of potash	200
	Hydrated lime	1,000

EXPERIMENTAL SERIES 2

Check	None	None
N	16 percent nitrate of soda	300
NP	16 percent nitrate of soda	300
	16 percent superphosphate	600
PK	16 percent superphosphate	600
	50 percent sulfate of potash	200
NPK	16 percent nitrate of soda	300
	16 percent superphosphate	600
	50 percent sulfate of potash	200
NPKCa	16 percent nitrate of soda	300
	16 percent superphosphate	600
	50 percent sulfate of potash	200
	Crushed limestone	2,000

[1] The symbols used for plot designations and in other parts of this paper have the following meanings: N=Nitrogen (N); P=phosphoric acid (P_2O_5); K=potash (K_2O); and Ca=lime hydrate or carbonate.

METHODS OF SAMPLING AND CHEMICAL ANALYSES

Crop samples were obtained from 45 experiments on 19 identified and certain unidentified soil series representing a total of 29 soil types. Each experiment consisted of 5 or 6 differently treated plots; consequently, 401 crop samples were obtained to determine their ash, nitrogen, phosphorus, potassium, and calcium content.

Pasture grass and mixed hay.—The yields, and also the samples of pasture herbage and mixed grass hay reserved for chemical analysis, were obtained from the fence-enclosed part of each of the experimental areas. The herbage in the pastures was composed of mixed grasses

and clover consisting largely of white clover and occasionally some red or alsike clover, the density of the clover depending upon the soil type and the fertilizers applied. The yields of pasture herbage and mixed hay were obtained by cutting two separate square-yard quadrates with a hand sickle. The remaining part of the enclosed area was then cut and the grass removed, except in a small section where the crop was allowed to grow to the hay stage of maturity. The stage of growth, which was the pasturing stage (before definite jointing of the grass occurred), rather than specific time intervals was selected for the pasture cuttings. After the green weight of the herbage on the quadrates was obtained, a representative sample was selected for chemical analysis. Care was observed to eliminate soil and other foreign materials from the samples, but no special effort was made to free the samples from dust or other mineral matter adhering to the herbage, or to segregate the different botanical species. The pasture samples were dried in an oven at 70° C.

As nearly as it is possible to determine in a stand of mixed grasses and clover, the blooming stage was selected for cutting the mixed grass hay. A representative sample of the hay, freed from soil and other foreign material, was selected for chemical analysis and dried in an oven at 70° C.

Alfalfa.—Two or three separate square-yard quadrates (the number depending upon uniformity of stand in the plot) were cut in each plot to determine yields and to obtain samples for analysis. The half-bloom stage of maturity was selected for cutting the crop. All samples saved for chemical analysis were carefully examined for stage of maturity, and those not strictly complying were rejected. Plants other than alfalfa were sorted out and discarded, but no attempt was made to determine alfalfa varieties. The hay samples were air-dried or dried in an oven at 70° C., whichever was more convenient.

Oats and Wheat Hay.—Samples for chemical analysis were obtained from two or three separate square-yard quadrates and cut at the milk stage of maturity. The samples of crop material selected for analysis were freed from weeds and plants other than oats or wheat and air-dried.

Chemical Analyses.—All the plant samples were ground in a Wiley mill and suitable portions were dried at 105° C. for 3 hours to determine the dry weight. The nitrogen, ash, phosphorus, and calcium content was determined by the official methods (1, *pp. 121–137*) for these constituents. The potassium content was determined by the cobaltinitrite method of Volk and Truog (7). All analytical results were calculated on the basis of dry weight of the plant materials.

EXPERIMENTAL RESULTS

Although the yields of the various crops used in the fertilizer experiments are recorded in the tables which follow, they will not be discussed in detail since this has been done elsewhere (5, 6). The fertilizers used for each of the two series of experiments were uniform in composition and applied in equal amounts. Their effect on like crops, therefore, should be relatively uniform, except for the influence of climatic factors and soil properties. Since certain soil properties of different soil types in the same soil series as well as soil properties

of different soil series may vary decidedly, and since the number of experiments established on any one soil series is small, some broad arbitrary grouping of soils and also of climatic conditions becomes necessary.

Both climatic factors and the parent material from which the soil is derived have a profound influence on inherent soil properties developed during the process of soil formation. A logical broad division of climatic conditions is the humid climate of western Washington and the relatively arid climate of central Washington. This grouping was applied to the experiments with alfalfa, the only crop grown on a substantial number of irrigated soils in central Washington and nonirrigated soils in the humid area of western Washington. The logical broad division of parent material from which the soils in western Washington are derived is to place the mineral soils in the glaciated area of northwestern Washington in one group, called for convenience soils derived from glacial material, and those in the nonglaciated area of southwestern Washington in - a second group, called for convenience soils derived from residual material. Some of the soils derived from alluvial material in both northwestern and southwestern Washington, however, may contain both glacial and residual material. A third group consists of soils derived from organic material. This grouping of soils was applied to the pasture grass and mixed hay experiments. No special grouping of soils or climate was made for the oat and wheat experiments.

COMPOSITION OF PASTURE GRASS, FIRST CUTTING

In all cases the pasture herbage consisted of mixed grasses and clover, largely white clover, the density of the clover depending upon soil type and fertilizer treatments. The analytical results and also the yields of 13 experiments on western Washington mineral soils are recorded in table 2. The data show wide differences in the composition as well as in the yields of herbage from plots treated with different fertilizers on the same soil type, and also of herbage produced on different soil types. The extreme values for ash ranged from 5.09 to 21.97 percent, for nitrogen from 1.33 to 3.92 percent, for phosphorus from 0.203 to 0.554 percent, for potassium from 0.63 to 4.40 percent, and for calcium from 0.27 to 1.59 percent.

In certain cases, like fertilizer treatments on different soils produced unlike effects which may have been accentuated by variations in meteorological conditions during different years. No attempt was made, however, to evaluate the influence of meteorological factors. The effect of these factors over a period of 3 or 4 years, the extent of the period of investigation, should have a tendency to be compensating rather than cumulative.

It is evident from the data in table 2 that the nitrogen and mineral content of the herbage produced on certain soils was consistently larger with and without fertilizers than that of herbage produced on certain other soils. This is noticeable also in the data for other forage crops shown in the tables which follow. Soil characteristics appear to have a pronounced influence on the composition of crops.

TABLE 2.—*Yield and composition of pasture grass (first cutting) as affected by different fertilizers and different soil types*

SOILS DERIVED FROM GLACIAL MATERIAL

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield (dry weight) per acre	Ash	N	P	K	Ca
		Number		*Tons*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
C-305	Puget silty clay	1	Check	1.39	7.96	1.99	0.316	0.99	0.59
			P	1.37	7.99	2.27	.375	.63	.68
			PK	1.35	9.18	2.29	.395	1.52	.61
			PKCa	1.27	9.37	2.36	.398	1.84	.70
			NPKCa	1.64	9.21	2.21	.358	2.21	.67
C-306	....do	1	Check	1.58	7.31	2.05	.318	2.75	.33
			P	1.61	6.93	2.00	.328	3.15	.34
			PK	1.97	8.53	1.80	.329	3.48	.35
			PKCa	1.81	8.10	1.90	.352	3.90	.35
			NPKCa	2.07	8.19	2.30	.334	4.21	.37
E-37	Bellingham silt loam.	1	Check	.87	6.71	1.39	.267	1.66	.29
			N	1.23	7.59	2.21	.316	2.65	.40
			NP	1.19	8.71	2.43	.359	2.04	.29
			PK	.97	7.33	1.33	.278	1.32	.28
			NPK	1.22	9.24	2.41	.435	3.15	.27
E-29	Custer silt loam	1	NPKCa	1.15	8.63	2.44	.387	2.81	.47
			N	1.00	11.19	2.24	.204	.71	.40
C-301	Bellingham silt loam.	1	Check	.84	8.86	2.48	.408	1.06	.66
			P	.85	9.83	2.42	.371	3.70	.50
			PK	1.22	9.61	2.24	.387	1.97	.50
			PKCa	1.33	9.89	2.52	.428	2.17	.82
			NPKCa	1.23	9.76	2.21	.403	2.09	.63

SOILS DERIVED FROM RESIDUAL MATERIAL

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield (dry weight) per acre	Ash	N	P	K	Ca
E-107	Chehalis loam	1	Check	0.63	21.17	2.30	0.282	2.04	0.43
			N	.55	19.25	2.10	.256	2.21	.54
			NP	.71	21.02	2.04	.290	1.89	.50
			PK	.61	18.20	2.41	.257	2.06	.59
			NPK	.62	21.97	2.53	.254	2.02	.73
			NPKCa	.92	17.32	2.58	.303	2.60	.73
E-55	Sacramento silty clay loam.	1	Check	.75	11.53	3.03	.312	1.83	1.45
			N	1.13	8.37	2.20	.280	1.45	.82
			NP	1.49	8.55	1.97	.361	1.23	.79
			PK	1.03	12.04	3.36	.357	3.10	1.59
			NPK	1.51	9.00	2.47	.411	2.74	.83
			NPKCa	1.71	11.69	1.99	.368	3.00	.80
D-118	Chehalis silty clay loam.	1	Check	.60	10.56	2.43	.433	3.23	.55
			N	.90	12.36	2.78	.521	3.55	.59
			NP	.60	12.84	3.04	.554	3.61	.74
			PK	.57	13.16	2.48	.484	2.81	.75
			NPK	.91	11.52	2.87	.532	3.68	.53
			NPKCa	.82	12.03	2.94	.491	3.34	.65
C-302	....do	1	Check	.79	10.98	2.20	.354	2.75	.64
			P	.72	10.89	2.57	.448	3.52	.64
			PK	.69	13.92	2.26	.397	3.00	.82
			PKCa	.88	13.37	2.48	.428	3.14	.81
			NPKCa	1.39	11.20	2.09	.406	3.81	.83
E-13	Salkum clay loam	1	Check	.38	11.49	2.03	.299	2.24	.71
			N	.72	7.55	2.83	.377	2.92	.80
			NP	1.00	8.86	3.55	.459	3.60	.66
			PK	.52	8.13	2.10	.347	2.22	.64
			NPK	1.23	9.51	3.34	.441	4.08	.61
			NPKCa	1.20	9.15	2.77	.384	3.05	.70
E-105	Olympic silty clay loam.	1	Check	.06	8.25	1.75	.203	1.47	.55
			N	1.16	8.89	3.56	.307	2.56	.47
			NP	.73	9.20	3.37	.393	2.82	.44
			PK	1.03	11.70	3.92	.508	4.40	1.14
			NPK	.97	9.15	3.44	.421	3.72	.53
			NPKCa	.61	11.23	3.55	.389	3.82	1.27
D-50	....do	1	Check	93	9.29	2.90	.340	4.02	.56
			N	1.21	9.42	2.91	.342	3.95	.62
			NP	1.32	8.96	2.60	.341	4.11	.58
			PK	.96	9.18	3.15	.303	3.96	.56
			NPK	1.85	8.63	2.51	.361	4.03	.67
F-16	Salkum clay loam	2	Check	.89	6.28	1.47	.291	2.30	.39
			N	3.29	5.40	1.98	.249	2.65	.36
			NP	3.46	5.09	1.93	.300	2.36	.32
			PK	1.02	5.94	1.47	.280	2.63	.34
			NPK	2.57	6.16	1.86	.315	2.75	.34

. On the basis of weighted averages, disregarding the effect of soil types and of meteorological conditions in different years, the herbage on the fertilized plots was benefited in that it contained increased percentages of nitrogen, phosphorus, and potassium, although not of ash and calcium, except possibly where lime was applied.   These
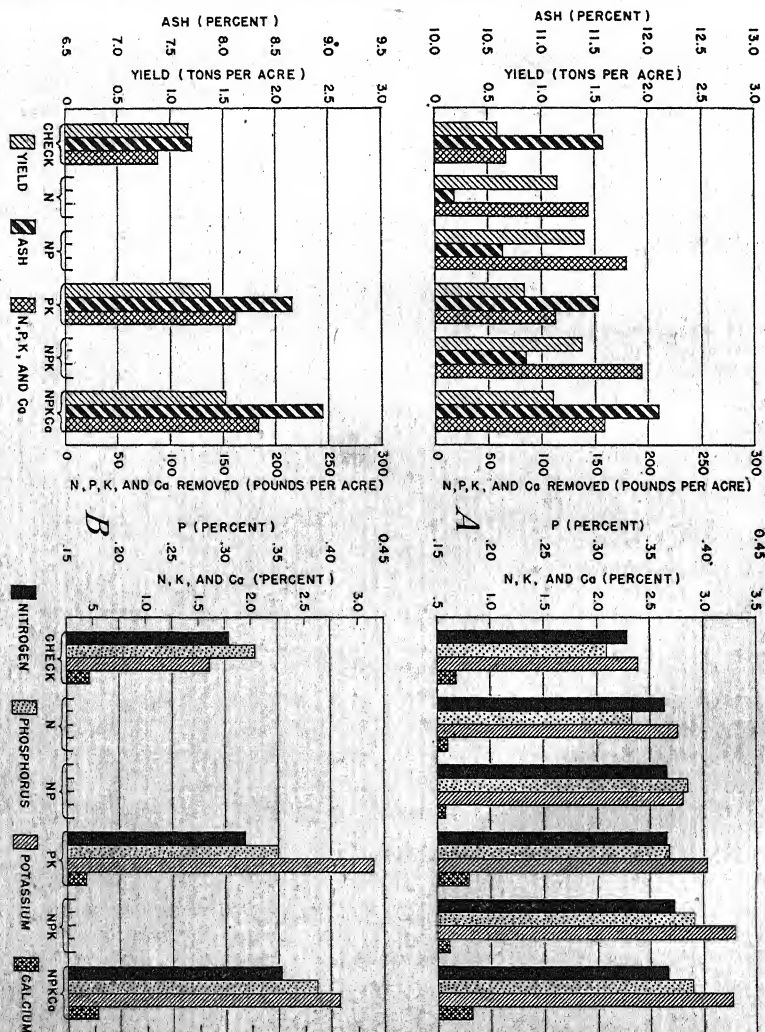
FIGURE 1.—Yield and composition of pasture grass, first cutting, on eight soils derived from residual material (*A*) and on four soils derived from glacial material (*B*).

findings are illustrated in figure 1 for eight experiments on soils derived from residual material, and four experiments on soils derived from glacial material.   The tendency of nitrogen fertilizers to cause an increased percentage of nitrogen in the pasture herbage is in agreement with the results of many investigations referred to in the review by Vandecaveye (*6*).   The tendency of phosphate fertilizers

to cause an increase in the phosphorus content of the herbage conforms with many of the results referred to in the above review and also with those reported by Fergus and Miller (*3*). Another interesting point indicated in figure 1 is that the herbage on the fertilized plots removed distinctly larger quantities of plant nutrients from the soil than did the herbage on the check plots. Evidently the increase in yields and in concentration of plant nutrients in the herbage resulted in a larger demand on the supply of plant nutrients in the soil.

In order to test the significance of the average values in figure 1 and subsequent figures an analysis of variance of the data appeared to be desirable, although no special experimental design for replication and randomization was planned for these experiments. The plots on soils derived from like parent material and receiving like fertilizers for like crops were considered as replicates and treated as one sample. It is conceivable that, regardless of the variability in soil types within the sample and the variability in meteorological conditions in different years, soil types derived from like parent material may possess a sufficient number of inherent characteristics in common to cause a definite trend in the effects produced by like fertilizers for like crops. An analysis of variance should be useful in ascertaining such trends, which in turn should be helpful in the interpretation of the experimental results. The summaries of the analysis of variance based on a 5-percent level of the *t* value are presented in separate tables for different crops. The summary for appropriate data pertaining to the first pasture-herbage cuttings is presented in table 3.

NITROGEN

Although the average values for nitrogen presented in table 3 for the first cutting of pasture herbage on eight soils derived from glacial material indicate substantial increases as a result of fertilizer applications, the values for the differences obtained by the analysis of variance on the 5-percent level are too small to be significant.

TABLE 3.—*Significance of differences in composition of pasture grass (first cutting) as indicated by analysis of variance (five-percent level of* t *value)*

Constituent	Means in percent of dry matter for treatments indicated					Difference required for significance			
	Check	N	NP	PK	NPK	All fertilizers	N fertilizer	P fertilizer	K fertilizer
N	2.16	2.57	2.62	2.53	2.68	0.87	0.95		
P	.303	.331	.382	.352	.396*	.087		0.123	
K	2.35	2.74	2.71	2.81	3.27	1.04			2.45
Ca	.62	.58	.54	.74	.56	.31			
	Means in percent of dry matter for soils indicated [1]								
	E-107	E-55	D-118	E-13	E-105	D-50	F-16	E-37	
N	2.28	2.61	2.72	2.77	3.21	2.82	1.74	1.96	.59			
P	.268	.344	.505	.385	.366	.337	.287	.331	.059			
K	2.04	2.07	3.38	3.01	2.99	4.01	2.54	2.16	.72			
Ca	.56	1.10	.63	.68	.63	.60	.35	.31	.21			
N	2.24	2.42	2.78	2.94	3.03	2.73	1.81	2.11		.50		
P	.271	.360	.501	.387	.381	.336	.297	.335			.064	
K	2.04	2.56	3.24	2.85	3.20	4.00	2.56	2.04				.82

[1] The values for different soils show significant differences among soils.
*=Significant increase.

It is evident that the effect produced by the fertilizers on different soil types varies widely. The analysis of variance of this effect indicates that a difference of 0.59 percent of nitrogen between the means for herbages of different soil types is significant. If the lowest mean value for the various soils shown in table 3 under "Difference required for significance," (first column) is used as a basis for comparison, it is found that the difference of 0.59 required for significance was exceeded in the majority of cases. Insofar as variations in nitrogen content of the first cutting of pasture herbage is concerned it appears that the effect of soil type was more pronounced in causing them than was the effect of fertilizers.

When the results of the plots receiving nitrogen-bearing fertilizers (omitting the results of the PK treatment from the statistical analysis) are compared with the results of the check plots, the values required for significance are slightly larger (table 3, under "Difference required for significance," second column), although the tendency indicated above where all the fertilizer treatments are included in the comparison is not altered appreciably. One of the reasons for the higher values required for significance when the PK treatment is omitted is that in most cases this treatment resulted in an increased proportion of clover to grasses, and consequently in a generally higher nitrogen content of the herbage.

PHOSPHORUS

The fertilizer treatments had a greater effect on the phosphorus content than on the nitrogen content of the pasture herbage. The difference in phosphorus required for significance between any two means for fertilizer treatments is 0.087 percent (table 3, under "Difference required for significance," first column). This value was exceeded for the NPK treatments and closely approached for the NP treatments as compared with the checks. Soil characteristics as expressed by soil types, however, also caused significant differences in the phosphorus content of the herbage. The value of 0.059 required for significance between any two means for soils is exceeded in the majority of cases when the lowest mean value for soils is used as a basis for comparison.

When the analysis of variance is confined to plots receiving phosphate-bearing fertilizers in comparison with the check plots the values for differences required for significance for both fertilizer and soil types are larger (table 3, under "Difference required for significance," third column). In this case the differences due to the effect of soil types are still significant, but the value of 0.123 for only those fertilizers that contained phosphates was not approached in any case. The relatively low average phosphorus content in the herbage obtained from the PK plots suggests that in the absence of nitrogen fertilizers the herbage had a tendency to absorb a smaller amount of the phosphorus applied as phosphates to the soil.

POTASSIUM

The average values in figure 1 show a definite increase in potassium content of the herbage produced on soils treated with fertilizers, and a further increase when potash was included in the fertilizers. The statistical data in table 3 for potassium reveal, however, that the

differences indicated by the average values are not significant. When the effect of soil type on the potassium content of the herbage is considered by the analysis of variance, it appears that this effect is significant, as in the majority of cases the difference required for significance was exceeded.

Calcium

The apparent differences in calcium content of pasture herbage, as indicated by the average values for calcium in figure 1, proved to be statistically nonsignificant insofar as they were affected by fertilizer treatments. As may be noted from the data on calcium in table 3, these differences are significant insofar as they are applied to soil types. Pasture grass produced on various soil types may be expected to contain significantly different percentages of calcium.

COMPOSITION OF PASTURE GRASS, SECOND CUTTING

The yields and analytical results for the second cutting of pasture herbage grown on five soils derived from glacial material and five soils derived from residual material are reported in table 4. The variations in composition and yields resulting from the application of different fertilizers, and from different soil types, were similar in character and magnitude to those for the first cutting. Like that of the first cutting the herbage of the second cutting contained larger percentages of nitrogen and minerals when produced on some soils than on others. The weighted averages illustrated in figure 2 show that in general the yields were considerably smaller, and consequently the total quantities of nutrients removed from the soil in the various experiments were less, as might be expected, for the second than for the first pasture cutting. The outstanding difference between the two cuttings with respect to chemical composition is that the percentages of ash, nitrogen, phosphorus, and potassium were smaller for the second cutting.

The effect of the fertilizers in general was manifested by an apparent increase in the avarage ash content of the herbage and an increased removal of total nutrients. The nitrogen fertilizers caused a larger average nitrogen content of the herbage on the soils derived from residual material, but not of the herbage on soils derived from glacial material. The phosphate and potash fertilizers produced an increased average percentage of phosphorus and potassium respectively in the herbage on both groups of soil. The average calcium content of the herbage was not affected regularly or appreciably by the various fertilizer treatments.

The significance of these apparent differences may be evaluated from table 5 in which the data suitable for the analysis of variance are summarized. Careful consideration of the influence of the fertilizers in general on the composition of the second cutting of pasture grass reveals no significant effect on the percentages of nitrogen, potassium, or calcium in the herbage. It appears, however, that the NP treatments resulted in a significant increase in the phosphorus content of the herbage, and that the PK treatments caused increases in phosphorus which approached significance.

Comparing the phosphate-bearing fertilizers and the potash-bearing fertilizers separately for their effect on the percentages of phosphorus

and potassium respectively in the herbage did not change the foregoing relationship to any significant degree. The value for the PK treatments suggests that the supply of phosphorus and potassium added to the soil by the phosphate and potash fertilizers was more effective for the second than for the first cutting.
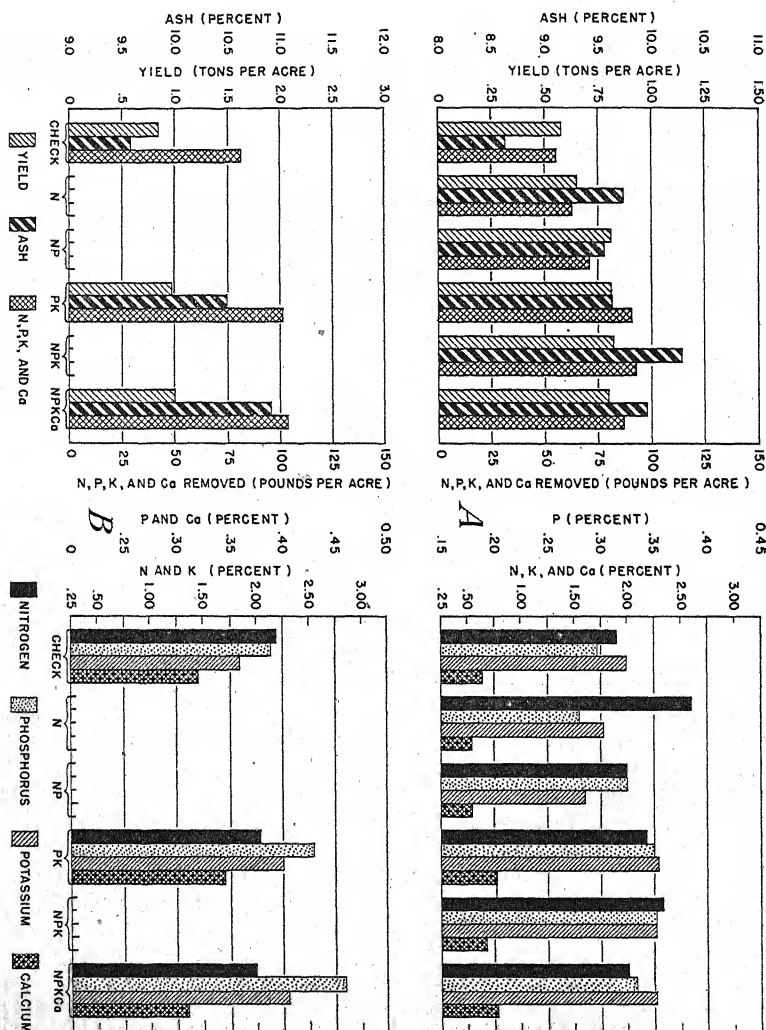
FIGURE 2.—Yield and composition of pasture grass, second cutting, on five soils derived from residual material (*A*) and on five soils derived from glacial material (*B*).

The statistical data pertaining to the effect of soil type on the composition of the second cutting of pasture grass indicate definitely that significant differences in the percentages of nitrogen, phosphorus, potassium, and calcium in the herbage resulted from differences in soil types.

TABLE 4.—*Yield and composition of pasture grass (second cutting) as affected by different fertilizers and different soil types*

SOILS DERIVED FROM GLACIAL MATERIAL

Soil No.	Soil type	Years ferti- lized	Fertilizer treatment	Yield (dry weight) per acre	Constituents in percent of dry weight				
					Ash	N	P	K	Ca
		Number		Tons	Percent	Percent	Percent	Percent	Percent
E-29	Custer silt loam	1	Check	1.72	6.42	1.18	.177	0.57	0.47
			N	2.44	4.49	1.10	.114	.37	.37
			NP	2.68	5.35	1.08	.203	.72	.34
			PK	1.35	6.71	1.14	.257	2.13	.52
			NPK	1.67	4.87	1.04	.181	.86	.27
E-37	Bellingham silt loam.	1	Check	.53	9.37	2.19	.300	2.52	.43
			N	.51	8.41	1.96	.325	1.74	.40
			NP	.66	9.13	1.76	.315	1.59	.30
			PK	.50	13.53	1.76	.319	1.89	.50
			NPK	1.04	8.69	1.62	.292	1.61	.31
			NPKCa	.98	7.67	1.43	.261	1.40	.35
C-301	Bellingham silt loam.	1	Check	.54	9.62	2.74	.489	1.99	.16
			P	.69	10.48	2.46	.498	1.68	.22
			PK	.82	10.01	2.65	.446	3.19	.22
			PKCa	.79	10.00	2.90	472	2.98	.16
			NPKCa	.64	10.80	2.33	.452	3.38	.21
C-305	Puget silty clay	1	Check	1.05	12.29	2.25	.372	2.14	.35
			P	.93	12.20	2.29	.433	2.36	.27
			PK	1.24	11.93	2.51	.479	2.48	.37
			PKCa	1.28	12.89	2.30	.443	2.80	.49
			NPKCa	1.36	13.65	2.17	.443	2.64	.50
C-306	....do	1	Check	.91	8.49	2.10	.460	1.38	.26
			P	1.11	9.62	2.07	.574	1.54	.29
			PK	.99	10.28	2.18	.629	1.65	.12
			PKCa	.98	11.13	2.32	.650	1.83	.22
			NPKCa	1.15	11.52	2.06	.680	1.82	.18

SOILS DERIVED FROM RESIDUAL MATERIAL

Soil No.	Soil type	Years ferti- lized	Fertilizer treatment	Yield (dry weight) per acre	Ash	N	P	K	Ca
E-107	Chehalis loam	1	Check	0.99	8.84	1.96	0.287	2.83	0.53
			N	.56	13.00	2.93	.310	2.49	.49
			NP	.95	11.80	2.28	.380	1.82	.47
			PK	.69	10.15	1.88	.370	2.74	.63
			NPK	.93	12.62	3.11	.420	2.67	.77
			NPKCa	.94	11.53	2.68	.373	2.68	1.51
E-55	Sacramento silty clay loam.	1	Check	.33	10.83	2.39	.282	.89	1.16
			N	.27	11.12	1.54	.264	.61	.81
			NP	.26	9.82	1.54	.290	.63	.72
			PK	.58	10.58	2.71	.304	1.46	1.39
			NPK	.40	12.90	2.18	.315	1.04	.98
			NPKCa	.23	10.62	1.72	.298	.79	.56
C-302	Chehalis silty clay loam.	1	Check	.75	8.70	1.59	.314	1.93	.16
			P	1.09	10.25	1.88	.366	2.55	.18
			PK	.97	10.41	1.54	.373	2.42	.19
			PKCa	1.13	10.33	1.67	.349	2.29	.29
			NPKCa	.95	10.79	1.56	.337	2.66	.24
E-13	Salkum clay loam	1	Check	.87	6.54	1.53	.286	2.05	.68
			N	1.30	5.76	1.81	.242	2.11	.41
			NP	1.44	6.41	2.19	.286	2.58	.66
			PK	.98	6.06	1.47	.238	1.65	.67
			NPK	1.30	6.64	1.65	.265	2.01	.54
			NPKCa	1.34	6.27	1.61	.274	1.84	.60
E-105	Olympic silty clay loam.	1	Check	.06	8.28	1.88	.325	2.35	.41
			N	.48	9.06	2.34	.304	1.89	.48
			NP	.61	10.19	2.00	.345	1.41	.36
			PK	.85	10.94	3.36	.465	4.25	.97
			NPK	.67	9.01	2.42	.407	3.39	.46
			NPKCa	.55	10.57	2.59	.387	3.41	1.00

TABLE 5.—*Significance of differences in composition of pasture grass (second cutting) as indicated by analysis of variance (5-percent level of t value)*

Constituent	Means in percent of dry matter for treatments indicated					Difference required for significance			
	Check	N	NP	PK	NPK	All fertilizers	N fertilizer	P fertilizer	K fertilizer
N	1.86	1.95	1.81	2.05	2.00	0.78	0.81	-------	-------
P	.276	.260	0.364*	.326	.313	.064	-------	0.074	-------
K	1.87	1.54	1.46	2.35	1.93	.99	-------	-------	1.94
Ca	.61	.49	.48	.78	.56	.23	-------	-------	-------

Constituent	Means in percent of dry matter for soils indicated [1]									
	E-107	E-55	E-13	E-105	E-29	E-37				
N	2.43	2.07	1.73	2.40	1.11	1.86	.66			
P	.353	.291	.263	.369	.186	.310	.054			
K	2.51	.93	2.08	2.66	.93	1.87	.84			
Ca	.58	1.01	.59	.54	.39	.39	.20			
N	2.57	1.91	1.80	2.16	1.10	1.88		.66		
P	.364	.298	.269	.386	.205	.307			.051	
K	2.78	1.13	1.90	3.33	1.18	2.01				.81

[1] The values for different soils show significant differences among soils.
*=Significant increase.

COMPOSITION OF THIRD PASTURE CUTTINGS AND OF PASTURE HERBAGE FROM ORGANIC SOILS

The prevalence of dry summers in western Washington restricts the growth of pasture herbage during the late summer months on mineral soils with good subsoil drainage because of lack of an adequate supply of soil moisture. In many cases growth was insufficient to justify a third cutting, and consequently only a limited number of third cuttings of pasture were available for analysis.

Two of the pasture experiments were on organic soils both of which yielded three cuttings. Since the parent material and other characteristics of organic soils differ distinctly from those of mineral soils, they were considered separately.

The yields and chemical composition of the third cuttings of the pasture herbage produced on mineral soils and of the three cuttings of the herbage on organic soils are reported in tables 6 and 7 respectively. The yields of the third cutting were small for both the mineral and organic soils. The chemical composition of the third cutting of the herbage varied in about the same order and magnitude as that of the first and second cuttings. No statistical analysis of the analytical data was made because the number of experiments involved was too small.

Organic soils frequently require drainage to lower the water table before they can be used successfully for crop production. A water table sufficiently low to permit normal tillage operations early in the spring, and sufficiently high to provide an adequate supply of moisture by subirrigation during the dry period in summer, is a distinct asset for pastures. Organic soils, therefore, should be especially suitable for pastures if the quality of the herbage they produce compares favorably with that of the herbage produced on mineral soils. Even though only two of the pasture experiments were on organic soils, a general com-

TABLE 6.—*Yield and composition of pasture grass (Third cutting) as affected by different fertilizers and different soil types*

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield (dry weight) per acre	Constituents in percent of dry weight				
					Ash	N	P	K	Ca
		Number		*Tons*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
E-105	Olympic silty clay loam	1	Check	0.12	11.63	1.48	0.243	1.69	0.58
			N	.24	8.53	2.96	.333	2.21	.55
			NP	.36	10.34	2.22	.405	2.06	.96
			PK	.91	10.81	3.22	.385	3.31	1.18
			NPK	.42	9.62	2.05	.342	2.04	.50
			NPKCa	.30	11.00	2.48	.355	2.95	1.34
C-305	Puget silty clay	1	Check	.37	11.04	2.45	.342	1.47	.95
			P	.36	10.74	2.79	.422	1.74	.85
			PK	.33	11.70	2.93	.432	1.58	.83
			PKCa	.37	12.01	3.45	.464	2.33	.73
			NPKCa	.42	13.12	3.17	.467	2.21	.78
E-29	Custer silty loam	1	Check	.12	9.38	2.03	.278	1.12	.77
			N						
			NP	.29	8.46	1.99	.338	1.08	.46
			PK	.21	10.45	2.05	.421	2.43	.85
			NPK	.54	9.20	1.92	.304	1.68	.46

TABLE 7.—*Yield and composition of pasture grass on organic soils as affected by different fertilizers*

FIRST CUTTING

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield (dry weight) per acre	Constituents in percent of dry weight				
					Ash	N	P	K	Ca
		Number		*Tons*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
C-303	Peat	1	Check	1.65	5.80	2.02	0.330	0.46	0.42
			P	1.66	5.75	2.10	.449	.28	.58
			PK	1.92	6.73	2.01	.415	1.06	.52
			PKCa	2.04	7.71	1.95	.384	1.44	.56
			NPKCa	2.22	6.73	2.10	.399	.43	.60
C-304	do	1	Check	1.49	5.06	2.47	.255	1.55	.54
			P	1.69	6.67	2.61	.403	1.38	.63
			PK	2.26	6.02	2.26	.325	2.18	.60
			PKCa	2.12	6.28	2.19	.329	2.84	.64
			NPKCa	2.84	6.03	2.34	.302	2.84	.59

SECOND CUTTING

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield per acre	Ash	N	P	K	Ca
C-303	Peat	1	Check	1.11	9.88	2.58	0.371	1.09	0.48
			P	1.05	8.81	2.48	.494	1.19	.34
			PK	1.30	10.13	2.63	.465	1.71	.27
			PKCa	1.25	11.18	2.38	.432	1.40	.69
			NPKCa	1.47	11.47	2.22	.418	1.10	.63
C-304	do	1	Check	.96	9.07	2.35	.254	.68	.64
			P	.97	8.57	2.80	.396	.55	.49
			PK	1.07	7.83	2.28	.332	1.06	.77
			PKCa	1.06	8.73	2.10	.327	1.61	.84
			NPKCa	1.40	8.91	2.00	.305	1.69	.82

THIRD CUTTING

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield per acre	Ash	N	P	K	Ca
C-303	Peat	1	Check	0.22	10.02	2.98	0.214	1.22	0.61
			P	.30	8.94	3.18	.243	1.15	.57
			PK	.27	9.11	3.22	.254	1.36	.63
			PKCa	.31	8.96	2.93	.228	1.29	.74
			NPKCa	.54	9.18	2.90	.226	1.10	.70
C-304	do	1	Check	.66	9.41	2.46	.213	4.58	.68
			P	.50	10.27	2.89	.308	5.55	.54
			PK	.82	11.24	2.17	.242	5.25	.64
			PKCa	1.03	9.39	1.93	.227	5.52	.66
			NPKCa	1.15	10.67	1.65	.208	5.05	.82

parison of the chemical composition of herbage produced on organic and mineral soils under similar climatic conditions should be of interest. On the basis of averages portrayed in figure 3 for the first two cuttings, the pasture herbage grown on organic soils had a tendency to be lower in percentage of ash, phosphorus, and potassium than that grown on the mineral soils (figs. 1 and 2), but the yields and nitrogen
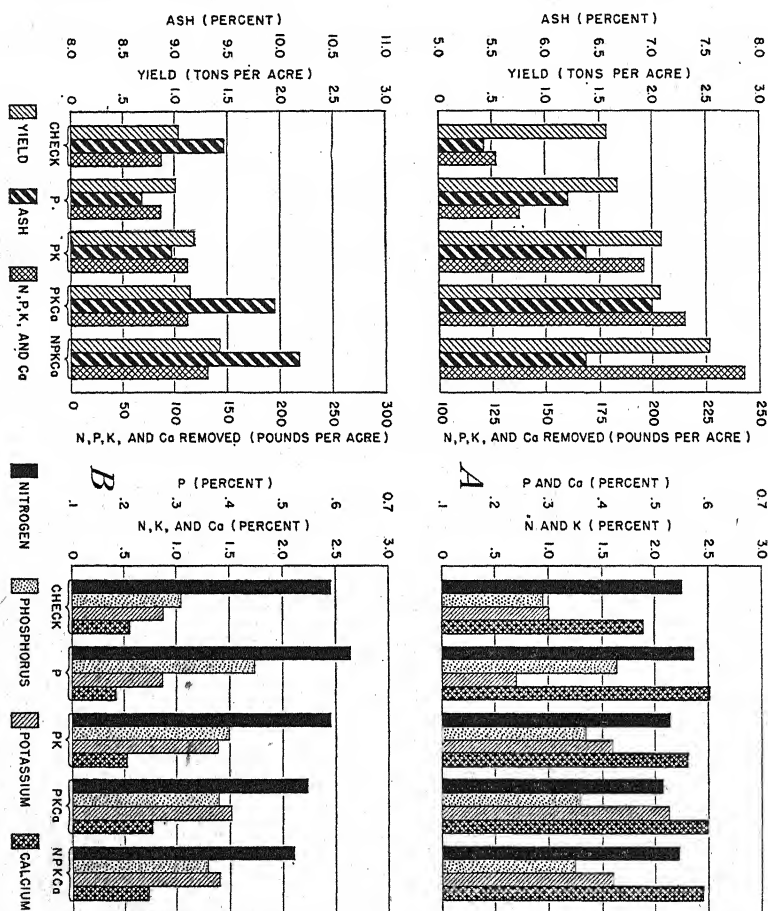
FIGURE 3.—Yield and composition of pasture grass on two organic soils: *A*, First cutting; *B*, second cutting.

content had a tendency to be higher for the herbage on organic soils. The effect of phosphate and potash fertilizers on the organic soils was especially good. It was clearly reflected in the markedly increased percentages of these constituents in the pasture herbage for both the first and second cuttings. With the exception of the P treatment, none of the fertilizers caused larger percentages of nitrogen in the herbage. The data in general indicate that with proper drainage and fertilizing practices many of the peat lands not now utilized for crop production in western Washington could be converted into excellent pastures.

COMPOSITION OF MIXED GRASS HAY

Mixed grass hay consisting largely of various grasses and some clover constitutes one of the principal winter roughages for livestock in western Washington, and therefore the quality of this hay as a livestock feed is important. The samples of hay selected for analysis were obtained from pastures or meadows established for some years, and were composed of different grasses mixed with various amounts of white clover and occasionally some red or alsike clover. The yields and chemical composition of the mixed grass hay samples from 10 experiments on soils derived from glacial material, 2 on organic soils, and 1 on a soil derived from residual material, or a total of 13 experiments, are recorded in table 8. The data show that the yields as well as the composition varied greatly. The extreme values for ash ranged from 4.06 to 10.08 percent, for nitrogen from 0.66 to 2.22 percent, for phosphorus from 0.091 to 0.500 percent, for potassium from 0.12 to 3.82 percent, and for calcium from 0.24 to 1.26 percent. Since the hay samples were cut at the same stage of maturity as nearly as could be determined, it is obvious that the wide differences in composition, and presumably in the quality of the hay as a livestock feed, were affected by the fertilizer treatments, and particularly, by soil properties.

TABLE 8.—*Yield and composition of mixed grass hay as affected by different fertilizers and different soil types*

SOILS DERIVED FROM GLACIAL MATERIAL

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield (dry weight) per acre	Ash	N	P	K	Ca
		Number		Tons	Percent	Percent	Percent	Percent	Percent
E-23	Bellingham silt loam	1	Check	1.54	5.38	1.63	0.273	1.11	0.75
			N	1.03	5.72	2.22	.251	1.28	1.02
			NP	1.57	4.69	1.35	.244	1.05	.36
			PK	2.46	6.01	2.09	.237	1.99	1.07
			NPK	1.69	6.24	2.06	.222	1.60	1.12
E-102	Quillayute loam	3	Check	1.11	4.72	.87	.250	1.89	.28
			N	1.87	4.76	.96	.233	1.67	.27
			NP	2.20	4.47	.98	.235	1.95	.24
			PK	1.92	9.08	2.02	.310	3.82	.88
			NPK	2.89	4.61	1.08	.235	2.28	.25
			NPKCa	3.61	5.12	1.10	.226	2.50	.40
E-1	Everett gravelly silt loam.	1	Check	1.23	4.38	1.01	.164	.68	.43
			N	2.18	5.33	1.48	.164	.95	.66
			NP	3.57	5.04	1.59	.244	1.70	.51
			PK	1.97	7.91	2.11	.274	3.41	.79
			NPK	3.27	6.63	1.52	.240	3.01	.52
			NPKCa	3.47	7.10	1.63	.267	3.08	.75
E-30	Lynden sandy loam.	1	Check	.75	4.67	1.32	.154	.90	.58
			N	1.72	4.06	1.69	.261	.57	.35
			NP	1.26	4.57	1.42	.164	.99	.58
			PK	2.02	5.56	1.86	.236	1.69	.82
			NPK	2.68	5.30	1.72	.219	2.20	.51
			NPKCa	1.99	5.51	1.57	.241	2.16	.61
C-22	Spanaway gravelly sandy loam.	1	Check	1.33					
			N	1.87	7.16	1.20	.158	1.56	1.26
			NP	2.13	5.68	1.03	.177	1.57	.83
			PK	1.06					
			NPK	2.67	5.27	1.04	.156	1.57	.64
			NPKCa	2.80	6.36	.94	.154	2.57	.75
C-301	Bellingham silt loam.	1	Check	1.71	9.55	1.33	.284	.84	.48
			P	2.40	9.22	1.50	.314	.56	.38
			PK	2.26	8.70	1.75	.296	1.01	.60
			PKCa	2.62	9.15	1.53	.290	1.07	.66
			NPKCa	2.55	9.07	1.12	.210	.71	.47

TABLE 8.—*Yield and composition of mixed grass hay as affected by different fertilizers and different soil types*—Continued

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield (dry weight) per acre	Constituents in percent of dry weight				
					Ash	N	P	K	Ca
			Number	*Tons*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
			Check	2.65	8.21	1.54	.133	1.42	.96
			P	3.08	8.13	1.37	.221	1.37	.91
C-305	Puget silty clay	1	PK	3.07	8.81	1.50	.172	2.23	.76
			PKCa	3.00	9.87	1.48	.244	2.42	1.16
			NPKCa	4.30	8.54	1.36	.174	1.84	.89
			Check	3.43	6.13	1.34	.410	3.05	.49
			P	3.92	6.36	1.14	.381	2.65	.49
C-306	do	1	PK	3.98	5.90	1.33	.313	2.17	.41
			PKCa	4.81	6.63	1.34	.500	3.41	.54
			NPKCa	3.63	8.38	1.39	.468	3.46	.56
			Check	4.50	6.62	.91	.159	1.44	.41
			N	4.58	7.40	1.18	.188	1.69	.29
E-117	Puget loam	1	NP	4.70	6.33	.96	.185	1.18	.27
			PK	3.63	6.97	.66	.166	1.28	.29
			NPK	4.71	7.20	.90	.186	1.12	.30
			NPKCa	5.13	7.81	.89	.197	1.06	.36
			Check	1.93	8.11	1.65	.234	2.72	.94
			N	2.08	6.84	1.39	.227	2.67	.60
E-47	Puget silty loam	1	NP	2.56	8.04	1.80	.282	2.89	.72
			PK	2.14	8.04	1.74	.300	3.12	.68
			NPK	3.32	6.83	1.72	.292	2.48	.42
			NPKCa	3.31	8.72	1.88	.338	3.40	.70

SOILS DERIVED FROM RESIDUAL MATERIAL

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield	Ash	N	P	K	Ca
			Check	2.65	8.87	1.30	0.245	1.85	0.25
	Chehalis silty clay loam		P	3.42	8.18	1.54	.286	1.96	.53
C-302		1	PK	2.58	9.13	1.62	.255	2.21	.95
			PKCa	2.45	8.78	1.24	.257	2.30	.95
			NPKCa	2.54	10.08	.95	.240	2.07	.89

SOILS DERIVED FROM ORGANIC MATERIAL

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield	Ash	N	P	K	Ca
			Check	3.31	7.77	1.41	0.268	1.14	0.55
			P	3.14	6.66	1.42	.358	.83	.73
C-303	Peat	1	PK	3.92	7.25	1.49	.310	1.78	.59
			PKCa	3.84	8.44	1.40	.289	1.83	.64
			NPKCa	4.10	8.50	1.68	.315	1.50	.80
			Check	2.24	7.66	1.65	.091	.12	.79
			P	2.24	6.99	1.86	.271	.73	.71
C-304	do	1	PK	3.38	7.78	1.67	.250	1.49	.61
			PKCa	2.88	6.55	1.52	.224	.82	.60
			NFKCa	3.38	6.96	1.42	.231	.85	.66

On the basis of averages as illustrated in figure 4 for nine soils derived from glacial material and for two organic soils, it appears that applications of suitable fertilizers can be expected to result in increased yields, and consequently in the removal of larger quantities of plant nutrient from the soil.

A comparison of the average values shown in figure 4 with those in figures 1 and 2 reveals that although the yields of dry matter produced by the hay crops were larger than those produced by the totals of the first two cuttings of pasture herbage, the average amounts of nitrogen, phosphorus, potassium, and calcium removed by the hay crops from either the mineral or organic soils, except for the nonfertilized and PK plots on mineral soils, were markedly smaller than the amounts removed by the combined first two cuttings of pasture herbage. Evidently pasture grass draws more heavily on the supply of plant nutrients in the soil than does the mixed hay. Furthermore, it is noted that the average percentages of ash, nitrogen, and phosphorus, al-

though not the average percentages of potassium and calcium, were decidedly smaller in the hay than in the pasture herbage. This explains why smaller quantities of plant nutrients were removed by the hay crops despite their higher yields.

The effect of fertilizers on the composition of hay was less pro-
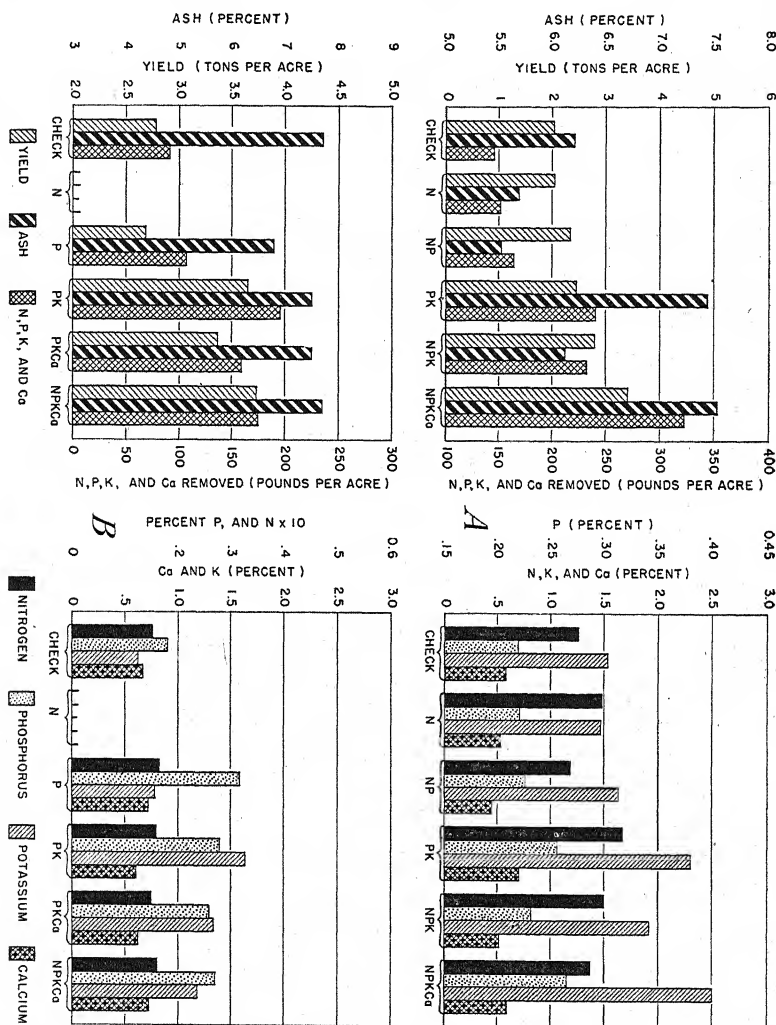
FIGURE 4.—Yield and composition of mixed hay on nine soils derived from glacial material (*A*), and on two organic soils (*B*).

nounced than it was on the composition of pasture herbage. The average values in figure 4 disclose a tendency for the fertilizers to produce a slight increase in the nitrogen content of the hay on the mineral soils, but not on the organic soils. The average percentage of phosphorus in the hay on the mineral soils was not increased appreciably by any of the fertilizers, except the PK and NPKCa treatments, but was

increased markedly by all the fertilizer treatments on the organic soils.

The significance of the apparent variations in composition of the mixed hay produced on six soils derived from glacial material may be evaluated from the summarized analysis of variance in table 9. These data show that with odds of 19:1 the larger percentages of nitrogen and potassium in mixed grass hay produced with PK fertilizers are significant. Perhaps the reason this treatment caused a significant increase in nitrogen content of the hay is that it resulted in a larger percentage of clover in the vegetation. The values obtained by evaluating the effect of the nitrogen-bearing fertilizers alone are not significant. Similarly, the values for percentages of phosphorus and potassium in the hay are not significant when the phosphate-bearing fertilizers and the potash-bearing fertilizers respectively are considered statistically. The data relating to the effect of soil types on the composition of mixed hay denote that variation in soil properties may be expected to result in significant differences in the percentages of nitrogen, phosphorus, potassium, and calcium in mixed grass hay grown on different soil types.

COMPOSITION OF ALFALFA HAY

For many years alfalfa has been widely grown on the irrigated semiarid land in central Washington, and in recent years persistent attempts have been made to introduce this crop extensively in western Washington. Good stands and satisfactory yields of alfalfa are not easily obtained on a large proportion of the western Washington soils, but because of the well-recognized high quality of alfalfa hay as a livestock feed, alfalfa production has increased steadily. Accordingly 7 of the 13 fertilizer experiments from which samples of alfalfa hay were obtained for analysis were on western Washington soils.

TABLE 9.—*Significance of differences in composition of mixed grass hay as indicated by analysis of variance (5-percent level of* t *value)*

Constituent	Means in percent of dry matter for treatments indicated					Difference required for significance			
	Check	N	NP	PK	NPK	All fertilizers	N fertilizer	P fertilizer	K fertilizer
N	1.23	1.49	1.35	1.75*	1.50	0.49	0.48	--------	--------
P	.206	.221	.226	.254	.232	.058	--------	0.069	--------
K	1.46	1.47	1.63	2.55*	2.12	.98	--------	--------	2.30
Ca	.57	.53	.45	.76	.52	.34	--------	--------	--------

Constituent	Means in percent of dry matter for soils indicated [1]									
	E–23	E–102	E–1	E–30	E–117	E–47				
N	1.87	1.18	1.54	1.60	1.92	1.66	.42	--------	--------	--------
P	.245	.253	.217	.207	.177	.267	.049	--------	--------	--------
K	1.41	2.32	1.95	1.27	1.34	2.78	.83	--------	--------	--------
Ca	.86	.38	.58	.57	.31	.67	.28	--------	--------	--------
N	1.82	.97	1.40	1.54	.99	1.64	--------	.31	--------	--------
P	.244	.257	.230	.143	.174	.277	--------	--------	.045	--------
K	1.57	2.66	2.37	1.60	1.28	2.77	--------	--------	--------	.97

[1] The values for different soils show significant differences among soils.
*= Significant increase.

The yields and chemical composition of the first cutting of alfalfa hay for 13 fertilizer experiments are recorded in table 10. The yield data denote that the alfalfa on the majority of the experimental soils responded favorably to fertilizer treatments. It is noted also that the chemical composition of the hay varied widely depending largely upon soil type. The weighted average values for yields and composition illustrated in figure 5 for the western Washington soils and the irrigated central
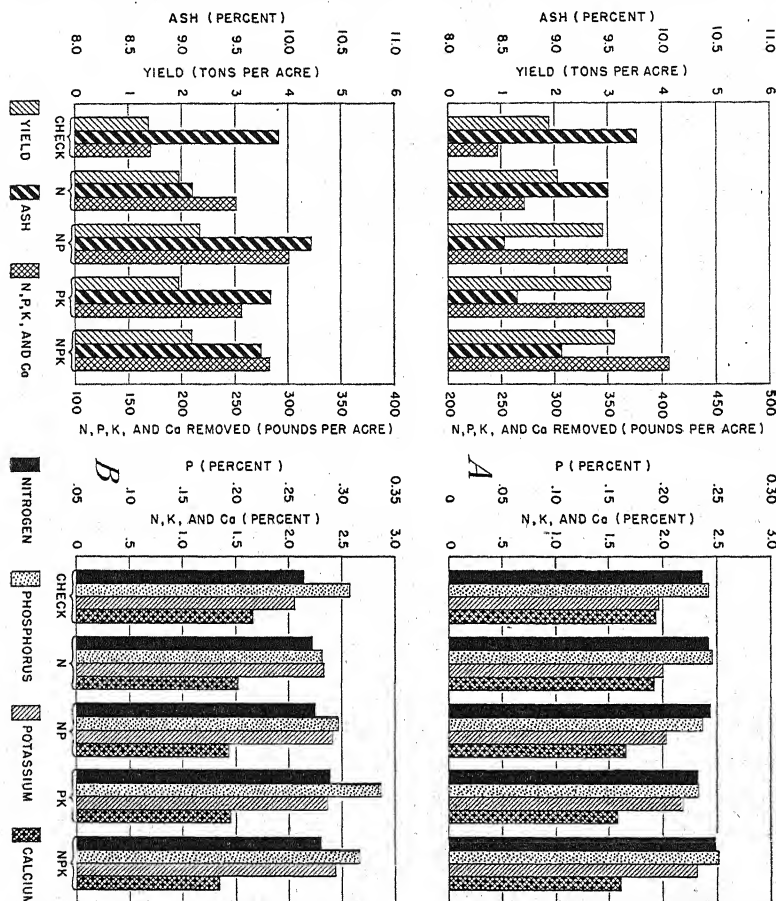
FIGURE 5.—Yield and composition of alfalfa hay, first cutting, on six arid irrigated soils (*A*) and on seven humid soils derived from glacial material (*B*).

Washington soils respectively indicate some interesting relationships. It may be observed that the average yields of hay and the corresponding quantities of plant nutrients removed from the soil were larger for the alfalfa on the irrigated soils than for that on the western Washington soils. The sum of the quantities of nitrogen, phosphorus, potassium, and calcium absorbed by the first cutting of alfalfa ranged from 170 pounds per acre for the nonfertilized plots on western Washington soils to 408 pounds per acre for the NPK plots on the irrigated soils. When

TABLE 10.—*Yield and composition of alfalfa hay (first cutting) as affected by different fertilizers and different soil types*

HUMID, NONIRRIGATED SOILS IN WESTERN WASHINGTON

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield (dry weight) per acre	Constituents in percent of dry weight				
					Ash	N	P	K	Ca
		Number		Tons	Percent	Percent	Percent	Percent	Percent
C-50	Dungeness silt loam.	1	Check	2.14	8.68	2.10	0.235	1.96	1.51
			N	2.14	9.78	2.20	.237	3.49	1.20
			NP	3.17	13.66	2.77	.363	3.32	1.58
			PK	3.43	9.40	2.52	.331	2.80	1.45
			NPK	3.22	10.07	2.28	.324	2.88	1.27
E-61	Puget silt loam	1	Check	1.62	8.11	2.22	.296	2.74	1.07
			N	1.76	8.17	2.19	.257	2.54	1.18
			NP	2.19	8.41	2.70	.293	3.10	.96
			PK	2.32	7.71	2.51	.302	2.85	.94
			NPK	2.09	7.69	2.72	.322	2.93	.89
E-56	do	3	Check	1.32	9.14	2.24	.278	1.73	2.03
			N	3.22	8.55	2.54	.274	2.39	1.41
			NP	4.18	8.57	2.36	.259	2.22	1.51
			PK	2.46	9.42	2.62	.328	2.39	1.64
			NPK	2.75	9.11	2.77	.284	2.31	1.54
C-67	do	1	Check	3.07	7.98	1.87	.274	1.99	.90
			N	3.83	8.56	2.15	.317	2.47	1.00
			NP	4.31	8.01	1.63	.263	2.26	.62
			PK	3.72	8.09	1.92	.269	2.07	.59
			NPK	4.51	7.44	2.00	.274	2.14	.81
C-153	Lynden fine sandy loam.	1 2	Check	.39	15.27	2.97	.259	.87	2.98
			N	.38	11.57	2.79	.229	.55	3.15
			NP	.44	15.70	2.80	.236	.79	2.81
			PK	.35	15.83	3.27	.314	1.55	2.51
			NPK	.48	16.12	3.01	.288	1.55	2.33
			NPKCa	.45	14.61	3.30	.318	1.55	2.37
C-43	Felida silt loam	1	Check	.43	10.87	1.85	.426	2.41	1.73
			N	.94	8.27	1.84	.314	2.32	1.46
			NP	.81	8.42	1.82	.311	2.68	1.26
			PK	.68	9.19	1.99	.446	2.75	1.48
			NPK	1.45	8.50	1.54	.342	2.58	1.23
C-71	Felida clay loam	1	Check	.68	9.42	1.81	.391	2.73	1.37
			N	1.53	8.88	1.91	.344	2.57	1.21
			NP	1.36	8.85	1.67	.354	2.54	1.25
			PK	.77	9.23	1.87	.367	2.09	1.54
			NPK	.94	9.30	1.74	.378	2.63	1.35

ARID, IRRIGATED SOILS IN CENTRAL WASHINGTON

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield	Ash	N	P	K	Ca
E-112	Dark sandy loam	1	Check	3.87	7.93	1.86	0.230	2.00	1.14
			N	3.49	7.71	2.03	.213	1.88	1.30
			NP	4.00	8.28	2.19	.229	1.83	1.45
			PK	3.74	7.35	2.01	.241	1.94	1.13
			NPK	3.41	9.79	2.41	.262	2.29	1.61
C-121	Sandy loam	1	Check	1.51	10.27	2.06	.241	2.20	2.44
			N	2.16	10.44	2.15	.258	2.29	2.34
			NP	2.59	10.18	2.35	.231	2.70	2.04
			PK	4.10	11.15	2.43	.237	2.77	2.59
			NPK	3.45	9.34	2.27	.207	2.19	1.99
E-106	Pasco sand	1	Check	1.57	11.15	3.59	.373	2.46	2.42
			N	1.49	10.55	3.34	.323	2.28	2.25
			NP	1.95	9.33	3.30	.332	2.60	1.79
			PK	1.67	9.68	2.83	.270	2.29	1.83
			NPK	2.69	8.91	3.21	.317	2.25	1.62
C-120	Sandy loam	2	Check	1.69	8.89	1.91	.204	1.51	1.96
			N	2.09	8.65	2.26	.257	1.97	1.78
			NP	4.31	6.28	1.96	.188	1.25	1.56
			PK	4.25	7.41	1.83	.214	1.90	1.26
			NPK	4.25	8.59	2.13	.259	2.61	1.42
B-118a	do	1	Check	1.79	8.46	1.81	.198	1.54	1.58
			N	2.38	9.30	2.15	.235	1.76	1.66
			NP	3.41	8.45	2.17	.223	1.68	1.56
			PK	3.15	8.19	2.15	.201	2.05	1.27
			NPK	3.67	7.67	2.20	.231	2.20	1.25
C-101	Winchester sand	1	Check	.98	11.90	2.96	.211	2.03	2.09
			N	.73	10.37	2.65	.189	1.79	2.21
			NP	1.08	8.61	2.64	.217	2.12	1.54
			PK	1.30	8.09	2.68	.232	2.15	1.39
			NPK	1.14	10.07	2.72	.228	2.40	1.75

1 Poultry droppings 2 years.

this is compared with the values for pasture herbage in figures 1 and 2, it is obvious that the combined yields of the first two cuttings of herbage on the western Washington soils were similar to corresponding yields of the first cutting of alfalfa hay on the western Washington soils, and that the quantities of plant nutrients absorbed from the soil by the two cuttings of pasture herbage were smaller than those absorbed by equal yields of the first cutting of alfalfa hay. In this comparison it is assumed that such atmospheric nitrogen as was utilized by the alfalfa and by the legumes in the pasture herbage passed through the soil. Thus, the fact that alfalfa draws more heavily than pasture grass on the supply of plant nutrients in the soil, and particularly the mineral nutrients, is clearly manifested.

The effect of various fertilizers on the chemical composition of alfalfa hay was not so pronounced as it was for pasture herbage. The average values in figure 5 show that the various fertilizers had an irregular effect on the ash content of the alfalfa, although they had a tendency to produce a lower percentage of ash in the alfalfa grown in the irrigated soils than in that grown on the nonirrigated soils. On an average the fertilizers had no appreciable influence on the nitrogen and phosphorus content of the alfalfa on the irrigated soils, but they did cause a slight increase in the percentage of nitrogen in the alfalfa on the nonirrigated western Washington soils. The PK and NPK treatments on the latter soils caused an apparent increase in the phosphorus content of the alfalfa. Larger average percentages of potassium and smaller percentages of calcium in the alfalfa hay resulted from the fertilizer treatments on both groups of soils.

Even though the average values in figure 5 indicate that some of the fertilizer treatments had a tendency to cause an increase in the nitrogen, phosphorus, and potassium content of the first cutting of alfalfa the summarized results of the analysis of variance reported in table 11 disclose that these apparent increases are not significant. The data in table 11 show further that the apparent reduction indicated in figure 5 for calcium content of this crop on both the irrigated and nonirrigated soils is not significant.

Second and Third Cuttings

Although yields of the third cutting of alfalfa were obtained from several fertilizer experiments, crop samples for chemical analysis were selected from only one of these experiments. The yields and analytical data, therefore, were included in table 12 with those of the second cuttings of alfalfa obtained from 10 experiments.

The yields of the third cutting of alfalfa were small. The yields of the second cutting, as may be noted by examining the data in figures 5 and 6, were less on the average than those of the first cutting. Noteworthy also is the fact that although the fertilizer treatments had an irregular effect on the ash content of the alfalfa, they resulted in the removal of large quantities of plant nutrients from the soil by the alfalfa in all except one case, the PK treatments on western Washington soils, for which the average yield was less than the average yields for the check plots. The quantities of plant nutrients absorbed by the second cutting, while less than those absorbed by the first cutting,

TABLE 11.—*Significance of differences in composition of alfalfa hay (first cutting) as indicated by analysis of variance (5-percent level of t value)*

Constituent	Means in percent of dry matter for treatments indicated					Difference required for significance			
	Check	N	NP	PK	NPK	All fertilizers	N fertilizer	P fertilizer	K fertilizer
N	2.25	2.32	2.34	2.36	2.38	0.35	0.44		
P	.278	.265	.269	.289	.286	.058		0.071	
K	2.01	2.18	2.24	2.28	2.38	.53			0.72
Ca	1.79	1.70	1.53	1.51	1.47	.36			

	Means in percent of dry matter for soils indicated [1]										
	Humid, nonirrigated soils										
	C–50	E–61	E–56	C–67	C–153	C–43	C–71				
N	2.37	2.47	2.51	1.91	2.97	1.81	1.80	.17			
P	.298	.294	.285	.279	.265	.368	.367	.028			
K	2.89	2.83	2.21	2.19	1.06	2.55	2.51	.26			
Ca	1.40	1.01	1.63	.78	2.76	1.43	1.34	.18			
N	2.34	2.46	2.48	1.91	2.89	1.76	1.78		.16		
P	.313	.303	.287	.270	.274	.381	.373			.027	
K	2.55	2.84	2.14	2.07	1.32	2.58	2.48				.17

	Arid, irrigated soils										
	E–112	C–121	E–106	C–120	B–118a	C–101					
N	2.10	2.25	3.25	2.02	2.10	2.73		.17			
P	.235	.235	.323	.224	.218	.215		.028			
K	1.99	2.43	2.38	1.85	1.85	2.10		.26			
Ca	1.33	2.28	1.98	1.60	1.46	1.80		.18			
N	2.12	2.21	3.36	2.07	2.08	2.74			.16		
P	.241	.229	.323	.216	.213	.222				.027	
K	2.08	2.39	2.33	2.01	1.93	2.19					.17

[1] The values for soils show significant differences among soils.

TABLE 12.—*Yield and composition of alfalfa hay (second and third cuttings) as affected by different fertilizers and different soil types*

HUMID, NONIRRIGATED SOILS IN WESTERN WASHINGTON, SECOND CUTTING

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield (dry weight) per acre	Constituents in percent of dry weight				
					Ash	N	P	K	Ca
		Number		*Tons*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
E–56	Puget silt loam	3	Check	0.84	8.87	2.29	0.243	1.61	2.36
			N	1.44	7.96	2.52	.259	2.39	1.54
			NP	1.27	7.54	2.34	.254	1.57	1.90
			PK	.76	9.62	2.40	.265	1.57	2.50
			NPK	1.93	7.23	2.57	.282	1.36	1.70
C–67	do	1	Check	1.69	8.41	1.86	.253	1.79	1.39
			N	2.49	8.88	2.74	.263	2.48	1.46
			NP	2.52	8.56	2.19	.240	1.72	1.16
			PK	1.75	8.46	1.96	.222	1.42	1.30
			NPK	2.57	8.10	2.28	.214	1.50	1.36
E–61	do	1	Check	.74	8.20	1.74	.167	2.13	1.43
			N	.56	7.48	1.76	.136	1.94	1.36
			NP	.89	7.58	2.60	.174	1.94	1.25
			PK	.75	8.66	2.22	.166	2.30	1.48
			NPK	1.19	8.29	2.57	.160	2.20	1.53
D–2	Lynden sandy loam.	2	Check	.08	9.00	1.79	.390	2.73	1.32
			N	.12	7.48	1.21	.235	2.29	1.28
			NP	.15	7.86	1.31	.244	2.46	1.11
			PK	.18	7.80	2.23	.219	2.71	1.03
			NPK	.18	8.29	1.70	.221	2.37	1.38
			NPKCa	.34	7.30	2.14	.236	2.57	.85

TABLE 12.—*Yield and composition of alfalfa hay (second and third cuttings) as affected by different fertilizers and different soil types*—Continued

ARID, IRRIGATED SOILS IN CENTRAL WASHINGTON, SECOND CUTTING

Soil No.	Soil type	Years ferti- lized	Fertilizer treatment	Yield (dry weight) per acre	Ash	N	P	K	Ca
			Number	*Tons*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
B-105	Winchester sand	1	Check	3.06	9.24	2.65	.194	2.37	1.57
			N	2.70	8.88	2.68	.203	2.34	1.50
			NP	3.50	8.71	2.78	.238	2.25	1.50
			PK	3.19	7.93	2.87	.240	2.40	1.26
			NPK	3.72	9.29	2.94	.223	2.94	1.43
C-120	Sandy loam	1	Check	1.51	8.05	2.29	.227	1.72	1.67
			N	1.74	9.00	2.66	.275	1.87	1.73
			NP	2.64	7.66	2.77	.267	1.49	1.44
			PK	1.73	8.14	2.79	.293	1.91	1.35
			NPK	2.39	8.38	2.70	.291	2.29	1.39
E-112	Dark sandy loam	1	Check	2.41	9.40	2.20	.205	2.04	1.81
			N	2.41	9.01	2.26	.218	1.88	2.05
			NP	2.85	9.30	2.36	.222	2.06	2.03
			PK	2.68	10.47	2.43	.244	2.23	2.35
			NPK	2.73	10.38	2.48	.241	2.63	1.32
E-106	Pasco sand	1	Check	.93	8.70	2.79	.277	2.43	1.46
			N	1.18	9.17	3.23	.266	2.89	1.32
			NP	1.31	9.10	3.02	.254	2.77	1.49
			PK	1.04	9.33	3.16	.273	2.72	1.68
			NPK	.84	8.01	3.10	.259	2.04	1.59
C-101	Winchester sand	2	Check	1.03	10.67	3.08	.224	2.54	1.79
			N	1.16	9.91	3.15	.218	2.78	1.60
			NP	1.61	10.37	3.61	.300	2.72	1.45
			PK	1.61	9.12	3.08	.247	2.73	1.50
			NPK	1.69	10.57	3.40	.294	3.46	1.42
C-43	Felida silt loam	1	Check	1.44	10.13	2.38	.429	2.79	1.28
			N	1.45	12.75	2.39	.340	2.34	1.43
			NP	2.03	9.70	2.25	.396	2.04	1.42
			PK	1.20	9.67	2.34	.385	2.89	1.36
			NPK	1.86	9.20	2.32	.318	2.45	1.36

HUMID, NONIRRIGATED, SOILS IN WESTERN WASHINGTON, THIRD CUTTING

Soil No.	Soil type	Years ferti- lized	Fertilizer treatment	Yield (dry weight) per acre	Ash	N	P	K	Ca
C-67	Puget silt loam	1	Check	0.51	2.13		0.175	1.66	2.31
			N	.92	2.15		.208	1.78	1.70
			NP	.92	2.22		.189	1.63	2.07
			PK	.78	2.46		.207	1.44	2.36
			NPK	.95	2.15		.164	1.33	1.90

were still large, emphasizing the fact already pointed out that alfalfa in comparison with other forage crops draws heavily on the supply of plant nutrients in the soil.

The response of the second cutting of alfalfa to the fertilizer treatments as manifested by the chemical composition of this crop differed from the response of the first cutting in that, as indicated in figure 6, all the fertilizers caused an increase in the average percentage of nitrogen in the alfalfa on both groups of soils, and a decided increase in the average percentage of phosphorus in the alfalfa grown on the irrigated soils of central Washington. The effect of the fertilizers on the potassium and calcium content of the second cutting of alfalfa was irregular.

The significance of the apparent differences in chemical composition illustrated in figure 6 is expressed in the summarized data for the

analysis of variance in table 13. These data show that the apparent increases in the nitrogen and phosphorus content of the second cutting of alfalfa hay, as indicated by averaging the results, are not significant. Both the data in table 12 and table 13 show that the influence of soil characteristics possessed by different soil types resulted in marked differences in the chemical composition of this crop.
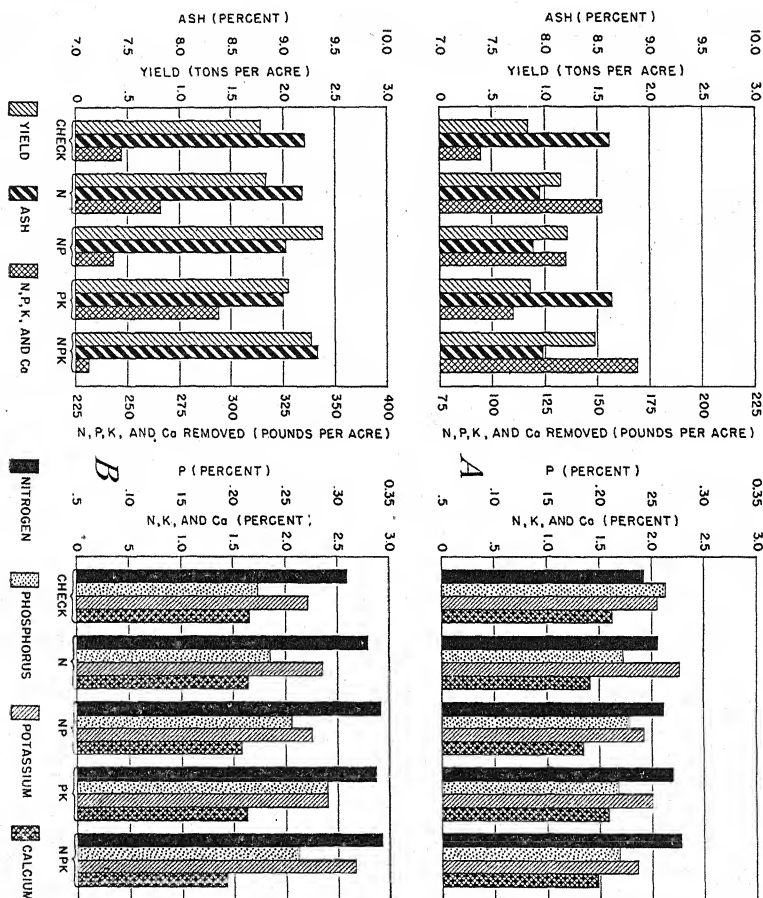
FIGURE 6.—Yield and composition of alfalfa hay, second cutting, from four humid soils derived from glacial material (*A*) and from five arid irrigated soils (*B*).

COMPOSITION OF OATS AND WHEAT CUT FOR HAY

Oats grown alone or as a mixture with vetch are roughages used for livestock in western Washington. Ordinarily the crop is cut in the early dough stage of maturity, and on soils of average fertility it returns better yields of hay than mixed grasses or clover. To a limited extent, wheat cut in the early dough stage is also used for hay in eastern Washington. The yield and composition data for oat hay obtained from six experiments are recorded in table 14, and the

yield and composition data for wheat hay from two experiments are given in table 16.

TABLE 13.—*Significance of differences in composition of alfalfa hay (second cutting) as indicated by analysis of variance (5-percent level of t value)*

Constituent	Means in percent of dry matter for treatments indicated					Difference required for significance			
	Check	N	NP	PK	NPK	All fertilizers	N fertilizer	P fertilizer	K fertilizer
N	2.29	2.43	2.52	2.54	2.56	0.39	0.49		
P	.253	.238	.253	.251	.242	.059		0.255	
K	2.16	2.27	2.06	2.21	2.23	.55			0.99
Ca	1.67	1.54	1.53	1.65	1.49	.37			

	Means in percent of dry matter for soils indicated [1]								
	Humid, nonirrigated soils								
	E-56	C-67	E-61	D-2	C-43	C-67a			
N	2.42	2.21	2.18	1.65	2.39	2.22	.21		
P	.261	.238	.161	.262	.374	.187	.031		
K	1.70	1.78	2.10	2.51	2.50	1.57	.29		
Ca	2.00	1.33	1.41	1.22	1.37	2.07	.20		
N	2.43	2.27	2.17	1.50	2.42	2.16		.21	
P	.261	.232	.166	.269	.382	.184			.108
K	1.51	1.57	2.21	2.60	2.71	1.48			.28

	Arid, irrigated soils								
	B-105	C-120	E-112	E-106	C-101				
N	2.78	2.64	2.35	3.06	3.26	.21			
P	.220	.271	.226	.266	.257	.031			
K	2.46	1.86	2.17	2.57	2.85	.29			
Ca	1.45	1.52	1.91	1.51	1.55	.20			
N	2.76	2.61	2.33	3.04	3.33		.21		
P	.224	.270	.228	.266	.266			.108	
K	2.57	1.97	2.30	2.40	2.91				.28

[1] The values for different soils show significant differences among soils.

OAT HAY

In view of the fact that much of the oat hay produced in western Washington is fed as a substitute for mixed grass hay, an evaluation of the yields and composition of these two forage crops with respect to their relative merits as livestock feeds is a practical consideration. A comparison of the data in tables 8 and 14 and in figures 4 and 7 reveals that in general the yield response of the oat crop to fertilizers and particularly to nitrogen-bearing fertilizers was better than that of mixed grass hay. The oat hay contained a considerably smaller percentage of ash and also smaller percentages of nitrogen, potassium, and calcium (but not phosphorus) than the mixed grass hay. Moreover, despite higher average yields the oat hay removed smaller amounts of plant nutrients from the soil than did the mixed grass hay. In considering the nutritive value of these two forages the relatively low protein and calcium content of oat hay may well be a factor that should serve to detract from its popularity as a winter roughage for livestock.

TABLE 14.—*Yield and composition of oats (cut for hay) as affected by different fertilizers and different soil types derived from residual and glacial material (nonirrigated land)*

Soil No.	Soil type	Years ferti-lized	Fertilizer treatment	Yield (dry weight) per acre	Ash	N	P	K	Ca
		Number		*Tons*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
E-12	Chehalis clay loam.	1	Check	1.09	5.76	0.96	0.249	0.57	0.24
			N	1.47	5.13	1.31	.173	.41	.22
			NP	2.91	5.47	1.11	.212	1.49	.32
			PK	.79	5.61	.99	.245	.41	.27
			NPK	1.98	5.05	1.00	.233	1.07	.23
			NPKCa	1.38	4.95	.93	.202	.92	.30
E-54	Hesson clay loam	1	Check	1.83	3.94	.81	.172	.63	.17
			N	2.73	3.81	1.14	.153	.83	.22
			NP	3.90	3.78	1.09	.162	.52	.22
			PK	2.16	4.33	.88	.182	.47	.12
			NPK	2.73	4.30	1.15	.184	.93	.23
D-31	Salkum silty clay	2	Check	2.18	4.95	.77	.400	1.19	.19
			N	3.04	6.24	.71	.327	1.39	.21
			NP	3.64	6.28	.94	.367	1.47	.21
			PK	2.91	5.74	.73	.239	1.58	.14
			NPK	3.73	5.59	1.15	.214	1.89	.20
D-12	Olympic clay loam	1	Check	.85	4.20	.70	.251	.59	.28
			N	3.41	4.20	.87	.153	.56	.27
			NP	3.84	3.66	.97	.281	.33	.25
			PK	1.71	4.44	.77	.258	.79	.45
			NPK	3.84	3.57	.77	.204	.67	.32
D-16	Colville silt loam	2	Check	1.95	7.49	1.46	.249	.20	.20
			N	2.37	8.10	1.41	.183	.27	.22
			NP	2.78	8.30	1.14	.254	.13	.25
			PK	2.95	8.21	1.46	.195	.74	.27
			NPK	2.54	7.68	1.30	.179	.38	.17
C-136	Orting loam	1	Check	3.35	5.15	.81	.240	1.45	.13
			N	5.23	5.19	.86	.252	1.66	.14
			NP	6.42	5.48	.82	.247	1.65	.13
			PK	3.20	6.72	.80	.440	1.65	.16
			NPK	7.43	5.15	.86	.243	1.56	.14

Unlike the mixed grass hay whose chemical composition was not affected uniformly or significantly by the application of various fertilizers, the oat hay appeared to be responsive to fertilizer treatments insofar as chemical composition is concerned. A definite increase in the average percentages of nitrogen and calcium, as well as of potassium, in the hay resulted from all the fertilizers applied. The average phosphorus content of the hay, however, was not increased by any of the fertilizers, and seemed to be decreased by the fertilizers which contained nitrogen. While nitrogen applied for this crop caused marked increases in yields, these increases were associated in most cases with reduced concentrations of phosphorus in the plant tissues.

The summarized results of the analysis of variance for the chemical composition of oat hay, recorded in table 15, reveal that although the trends of the average values represented in figure 7 are definitely in favor of higher percentages of nitrogen, potassium, and calcium in oat hay as a result of fertilizer applications, these apparent increases are not significant. When the effect of different soil types on the composition of oat hay is taken into account in evaluating the influence of fertilizer treatments, as shown in table 15, it appears that soil properties had a more potent influence on the composition of oat hay than had the fertilizer treatments.

WHEAT HAY

A careful examination of the yield and chemical composition data in table 16 for wheat hay produced on two central Washington soils under irrigation reveals certain outstanding characteristics when compared with the corresponding average values for oat hay in figure 7.
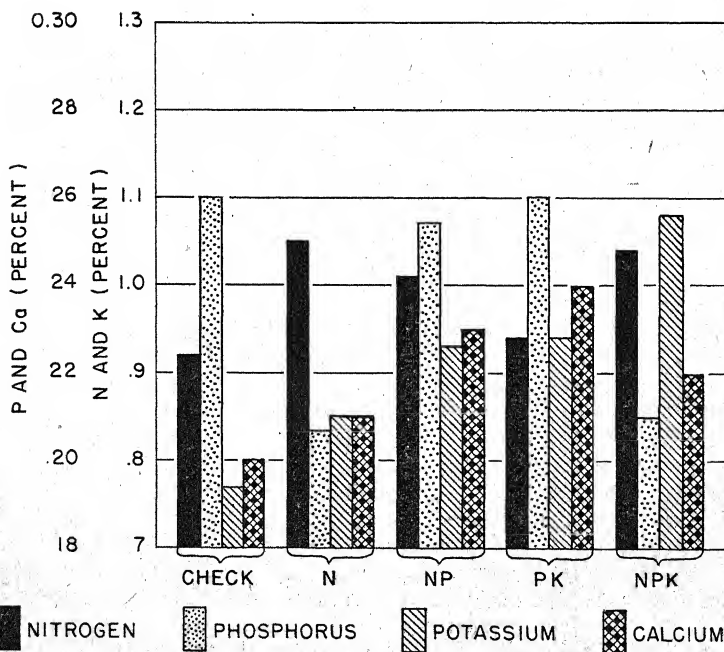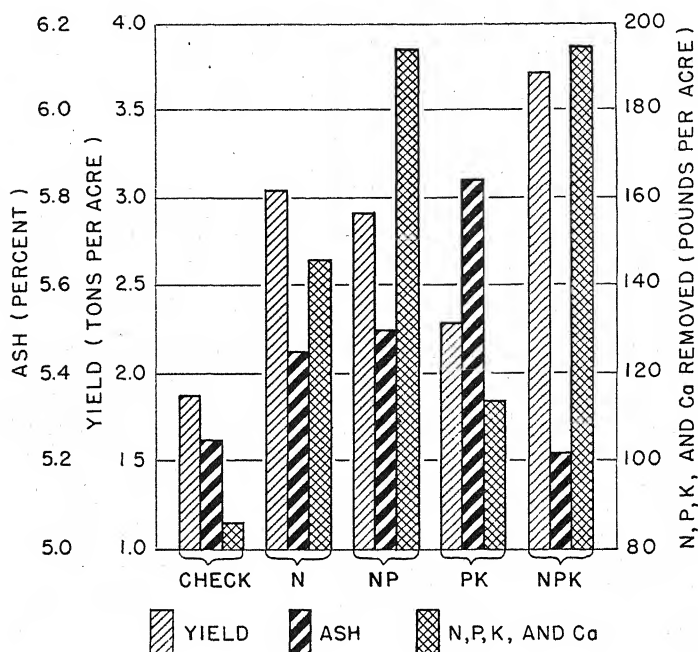
FIGURE 7.—Yield and composition of oat hay grown on six different soils.

TABLE 15.—*Significance of differences in composition of oats cut for hay as indicated by analysis of variance (5-percent level of* t *value)*

Constituent	Means in percent of dry matter for treatments indicated					Difference required for significance			
	Check	N	NP	PK	NPK	All fertilizers	N fertilizer	P fertilizer	K fertilizer
N	0.92	1.05	1.01	0.94	1.04	0.24	0.32	------	------
P	.260	.207	.254	.260	.210	.095	------	0.132	------
K	.77	.85	.93	.94	1.08	.46	------	------	0.77
Ca	.20	.21	.23	.24	.22	.08	------	------	------

	Means in percent of dry matter for soils indicated [1]									
	E-12	E-54	D-31	D-12	D-16	C-136				
N	1.07	1.01	0.86	0.82	1.35	0.83	.20	------	------	------
P	.222	.171	.309	.229	.212	.284	.073	------	------	------
K	.79	.68	1.50	.59	.34	1.59	.35	------	------	------
Ca	.26	.19	.19	.31	.22	.14	.07	------	------	------
N	1.09	1.05	.89	.85	1.33	.84	------	.21	------	------
P	.235	.175	.305	.248	.219	.292	------	------	.087	------
K	.68	.68	1.55	.68	.44	1.55	------	------	------	.32

[1] The values for different soils show significant differences among soils.

TABLE 16.—*Yield and composition of wheat as affected by different fertilizers and different soil types developed under the arid climate of central Washington (irrigated land).*

Soil No.	Soil type	Years fertilized	Fertilizer treatment	Yield (dry weight) per acre	Constituents in percent of dry weight				
					Ash	N	P	K	Ca
		Number		*Tons*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
E-110	Sandy loam	1	Check	5.61	7.98	0.96	0.143	0.27	0.09
			N	6.48	9.47	1.00	.084	.15	.10
			NP	7.96	8.43	1.04	.113	.25	.07
			PK	6.63	8.56	.80	.089	.54	.07
			NPK	7.29	7.57	.99	.132	.34	.09
E-111	Sandy gravelly loam.	1	Check	5.68	10.72	1.02	.156	.49	.13
			N	3.97	9.81	.94	.159	.30	.10
			NP	5.26	10.94	.82	.158	.32	.10
			PK	4.13	10.36	.79	.170	.27	.10
			NPK	4.86	11.45	.89	.154	.36	.09

The yields of wheat hay were exceptionally large and much above the average yields of the oat hay grown on western Washington soils. The ash content of the wheat hay, while not exceptionally large, was considerably larger than the average values for ash in oat hay. The nitrogen content of the wheat hay did not vary greatly from the average values for nitrogen in the oat hay, but the percentages of phosphorus, potassium, and calcium in the wheat hay were markedly lower than the average values for these constituents in the oat hay. As a matter of fact, the content of phosphorus, potassium, and calcium in the wheat hay was exceptionally small, and was not affected significantly by any of the fertilizer treatments on either of the two soils, although the fertilizer treatments caused marked increases in the yields of hay on one of the soils. These differences in the chemical composition of wheat and oat hay while rather outstanding may not be

significant from a practical standpoint, because of the limited number of experiments with wheat hay. Conclusions, therefore, are hardly justified until considerably more experimental evidence is obtained from wheat on different soil types.

CONCLUSIONS AND SUMMARY

When the farmer uses commercial fertilizers, his chief interest is focused on the probability of obtaining sufficient increases in crop yields to secure profitable returns from the extra money expended, and also on the probability of improving the quality of his crops. Any increase in the percentage of protein, phosphorus, or calcium that forage crops may contain as a result of fertilizer applications may be considered to be an improvement in the quality of these crops as live-stock feeds.

Four hundred and one forage-crop samples obtained from 45 fertilizer experiments on 29 different soil types were analyzed for their ash, nitrogen, phosphorus, potassium, and calcium content to determine the effect of different fertilizer treatments and soil types on the chemical composition. Certain features which may have significance with respect to the nutritional value of forage crops were revealed by this study.

Undoubtedly variations in meteorological conditions in different years contributed to the differences in the composition of the forage crops. It was not possible to measure the influence of meteorological factors in this particular experimental plan, but had they been measured it is doubtful whether the general results would have been altered appreciably, for no abnormal seasons occurred during the experimental period and the effect of commonly recurring variations in climatic factors have a tendency to be compensating rather than cumulative over a period of several years. Moreover, not all soil types are affected alike by variations in meteorological factors. A wet growing season may benefit crop growth on light soils with excessive drainage and be harmful to crop growth on heavy soils in which drainage is restricted. A dry growing season may have contrary effects.

On the basis of the experimental results there can be no doubt that the nitrogen, phosphorus, potassium, and calcium content of forage crops is affected markedly by differences in soil types. The effect of fertilizers on the composition of these crops is less certain. Since good responses from fertilizers were obtained on certain soil types in each group used for statistical analysis and not on others, it seems that the variability in soil characteristics was too large or the number of soils involved too few to serve as an indication that the changes in composition of the forage crops as affected by fertilizer applications are significant except in a few cases.

Like fertilizers applied to different soils produced markedly different effects on the protein, phosphorus, potassium, and calcium content of forage crops. Certain forage crops were more responsive to fertilizers than others. Pasture herbage proved to be the most responsive, indicating that the effect of fertilizers on the composition of forage crops is more pronounced in the early than in the more advanced stages of growth.

In the majority of cases applications of nitrogen and phosphate fertilizers supplying about 40 to 50 pounds of nitrogen (N) and 80 to

90 pounds of phosphoric acid (P$_2$O$_5$) per acre, and complete fertilizer supplying 80 to 100 pounds of potash (K$_2$O) per acre in addition to the amounts of nitrogen and phosphoric acid mentioned caused marked increases in the protein and phosphorus content of pasture herbage produced on soils in the areas represented by the experiments. With minor exceptions the complete fertilizer under these conditions resulted also in a larger potassium content of the herbage.

The response of mixed grass hay to these fertilizers was similar in character to that of the pasture herbage, but in a less pronounced degree. The general effect of mixtures of phosphate and potash fertilizers was to increase the amount of clover in mixed grass hay and consequently to bring about appreciably larger percentages of protein, phosphorus, and potassium in the hay.

The chemical composition of alfalfa and oats at the hay stage of maturity was less influenced by fertilizer treatments than was the chemical composition of pasture herbage or of mixed grasses in the hay stage of maturity, although the trend of the response of the former crops was in the same direction as that of the latter.

The calcium content of the forage crops was affected markedly by differences in soil types, but did not appear to be influenced to any significant degree by fertilizer treatments, except that applications of complete fertilizers for alfalfa resulted in a reduced calcium content of the hay.

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STUDIES OF MICROBIAL ACTIVITY, CHLORATE REDUCTION, AND CHLORATE TOXICITY IN SOILS TREATED WITH SODIUM CHLORATE [1]

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INTRODUCTION

One of the most important herbicides used in the control of perennial weeds is sodium chlorate. Its efficiency depends largely upon its persistence in the soil in a soluble unchanged condition until it is absorbed by the roots of growing plants. The rate of chlorate application does not foretell the effectiveness of a treatment since decomposition and leaching occur at different rates under different conditions. The factors playing the most important role in causing rapid chlorate reduction are undetermined. Such factors as temperature, moisture, organic matter, nitrates, soil reaction, biological activity, and the chemical and physical changes during the wetting and drying of soils have been reported to influence chlorate reduction or its apparent toxicity (*1, 2, 3, 8, 10, 11, 15*).[3]

That sodium chlorate might affect soil micro-organisms as well as higher plants has also been suggested. Since only a limited amount of work had been reported on this phase of the subject (*1, 2, 8, 12*), the writer, in association with cooperating agencies on weed research at the University of Minnesota, undertook a study of microbial populations and microbial activity in chlorate-treated soils. The studies included (1) effect of chlorate on soil micro-organisms, (2) toxicity of chlorate to plants, (3) influence of plant growth on microbial levels, (4) relation of microbial activity and aeration to chlorate reduction, and (5) effect of fertilizers on chlorate toxicity to plants.

LITERATURE REVIEW

Crafts (*3*) studied several soils under greenhouse conditions and found that increasing the mineral nutrient level of the soil invariably decreased chlorate toxicity. The addition of nitrates proved especially effective in both soil and nutrient cultures. He reported that chlorate absorption appeared to be a function of root activity and of the concentration and physical and chemical properties of the anions in the culture medium, and that under low nitrate conditions the plant, to its own detriment, will take up chlorate against a concentration gradient.

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Rosenfels and Crafts (*14*), studying chlorate toxicity in three different soils, again found that plants grew better on portions of soil in which the nitrates were concentrated. However, they gave no indication of the amount of chlorate reduction that might have occurred at the various nutrient levels during the month the plant seedlings were growing. Undoubtedly they assumed this factor to be negligible, or equal, for all portions regardless of the nutrient level.

Schwendiman (*15*) made studies to determine the relative importance of soil organic matter, soil temperature, and soil moisture as factors affecting chlorate toxicity and decomposition. He found that chlorate decomposition was greatly accelerated by high temperatures in combination with high moisture levels. Furthermore, he found increased catalytic power to be associated with an increase in chlorate decomposition, except under conditions of relatively low moisture and alternate wetting and drying of the soil. In this case the decomposition of chlorate was attributed to chemical and physical factors operating during wetting and drying of the soil. Otherwise the increments in moisture were believed to accelerate the activities of micro-organisms which are then responsible for the greater catalytic activity and the corresponding increase in chlorate decomposition.

Schwendiman attributes the lower toxicity in the presence of organic matter primarily to increments in nitrate content. The only time the rate of decomposition was roughly proportional to the amount of organic matter occurred under conditions of sustained complete soil saturation or when the treated soils were autoclaved.

Åslander (*1*) also mentioned that decomposition of chlorate is probably due to micro-organisms and that it occurs in soils supersaturated with water. He observed species of *Penicillium*, *Aspergillus*, and *Fusarium* together with numerous bacteria growing on top of a hay infusion medium in the presence of tenth normal sodium chlorate. Sodium chlorate did not retard ammonification or nitrification the spring following a fall application. Sodium chlorate is reported as not being detrimental to protozoa or to earthworms.

Bowser and Newton (*2*) found sodium chlorate did not prevent, or greatly affect, nitrification. Nitrification proceeded rapidly in the presence of chlorate when nitrogenous organic matter was added to the soil. In controlled laboratory tests sodium chlorate showed some depressing effect on bacteria and fungi as determined by plate counts.

Wilson, Crim, and Larson (*20*) reported that chlorates were most effective in killing perennial weeds when applications were made during July and August. Hulbert, Remsberg, and Spence (*9*) and Brown [4] found that late-summer and fall applications of chlorate were more effective than spring or midsummer applications and that more chlorate reached the subsoil and persisted for a longer period when applications were made during the fall.

MATERIALS AND METHODS

The studies on microbial activity were begun in the summer of 1940 at the Weed Experiment Station, Lamberton, Minn. At that time no facilities were available for conducting counts of micro-organisms by the dilution method. It was believed, however, that the relative numbers of organisms in soils from various plots might be compared

[4] BROWN, W. J. N. SOME SOIL MOISTURE RELATIONSHIPS IN FIELD BINDWEED (CONVOLVULUS ARVENSIS) CONTROL. Unpublished master's thesis. [Copy on file at the University of Minnesota.] 1942.

on the basis of gas production resulting from their attack on added carbonaceous materials. Dextrose was chosen for the material to be added since it is readily used by a broad group of organisms.

The method employed may be outlined as follows: One hundred grams of soil plus dextrose and water was placed in a 125-cc. Erlenmeyer flask. The flask was then stoppered with a one-hole rubber stopper with an inserted glass tube. Over the outer end of the glass tube was placed a short, flexible, heavy rubber tube and this in turn was closed by means of a screw clamp. The flask was then placed in a constant-temperature water bath. Pressures produced by gases resulting from microbial activity were measured by connecting the flask to a manometer made from capillary glass tubing and releasing the screw clamp. After the measurement the gases were released from the flask and the screw clamp tightened again. Additional readings were similarly taken at stated intervals.

The level of microbial activity in the various soils was determined on the basis of the amount of gas produced, measured by pressure, during the period of incubation. The incubation period and temperature used were approximately 86 hours and 27° C., unless otherwise recorded. The sum of three or more successive readings per sample, taken after 62, 74, and 86 hours of incubation, was used in determining the relative microbial activity of the soils examined.

The soil at Lamberton, Minn., from which samples were taken, has been designated as Clarion silt loam. All samples for comparing microbial activity were taken from the upper 6 inches of soil after the surface debris had been removed. A 3-inch pipe sharpened at one end was driven into the soil to obtain and remove uniform soil cores. Nine such cores were taken at random and then composited for each plot sampled. This composite sample was thoroughly mixed and a portion of it sifted and used. Usually three separate 100-gm. samples were taken from the sifted portion for the determination of microbial activity.

During the winter of 1940–41, controlled experiments were carried out at the University of Minnesota. In these experiments uniform soils obtained from greenhouse storage bins were used. Two series of 100-gm. samples of air-dry soil were placed in glass tumblers and treated with sodium chlorate at two different rates. The soils were incubated at room temperature with the moisture maintained at one-half the water-holding capacity of the soil. Bacterial counts were made according to the technique of Waksman (18). Sodium albuminate agar prepared as described by Waksman and Fred (19) was used. Ten plates per dilution were poured. Occasionally some plates could not be counted because of the prevalence of molds. In no case were plates counted that had an appreciable growth of mold. The colonies of *Actinomycetes* were included in the counts of bacteria. Two determinations were made of the number of fungi by the plate method; a dextrose-tartrate medium was used.

In the determination of the number of algae Bristol-Roach's method (18) was used. Ten replications of each decimal dilution and the tables of probability of Halvorson and Ziegler (6, 7) were employed. Tests for determining significant differences on counts of bacteria and fungi were made according to the t test of Fisher (4).

The method used for the quantitative chlorate analysis was that developed in the section of agricultural botany, University of Minne-

sota. This method has been used successfully for several years. It does not differ greatly in principle from the method employed by Bowser and Newton (*2*). A summary of the procedure used in making the analyses follows:

Place 100 gm. of air-dry soil in a bottle (300-cc. capacity or more), add 100 cc. of water, and shake (on shaker) for 30 minutes. Then add 10 cc. of 2 percent solution of potassium aluminum sulfate as a precipitant, and centrifuge for 20 minutes. Carefully pipette off a 25-cc. aliquot and put into a 250-cc. Erlenmeyer flask. To this add 25 cc. of 0.1 N (need not be exact but sufficient to reduce chlorate in aliquot) ferrous ammonium sulfate ($Fe(NH_4)_2$ $(SO_4)_2.6H_2O$) and boil gently 5 to 10 minutes. Cool to room temperature and add 10 cc. of Zimmermann-Reinhardt solution (*17, p. 607*). Titrate the remaining ferrous sulfate with potassium permanganate solution of a known normality. Each day boil at least two water blanks (25 cc.) plus 25 cc. of the ferrous ammonium sulfate solution, cool, add Zimmermann-Reinhardt solution, and titrate for checks on the normality of the reducing solution, which may change from day to day. Then obtain the sodium chlorate present in a given sample from the difference in titer.

$$\frac{\text{Ml. for check—ml. for any sample}}{1,000} \times \text{normality of } KMnO_4 = \text{equivalents of } ClO_3.$$

$$\text{Equivalents} - ClO_3 \times \frac{106.45}{6} \times \frac{110}{25} = \text{weight of sodium chlorate per 100}$$
gm. of air-dry soil.

In determining the smaller amounts of chlorate in these experiments, solutions nearer 0.05 N were used. As a preservative, 20 cc. of concentrated chemically pure H_2SO_4 was added per liter of ferrous ammonium sulfate solution. A qualitative test for chlorate, which was found to be helpful in certain cases, was also used. The soil solution was tested on a spot plate by adding one to several drops of a diphenylamine indicator solution which was made of 0.5 gm. diphenylamine, 5.0 cc. concentrated H_2SO_4, and 20 cc. orthophosphoric acid. If chlorate was present, a blue color usually appeared immediately. In concentrated sulphuric acid, nitrates color diphenylamine, though there seems to be a safe distinction between the coloration caused by nitrates and that caused by chlorate even if the concentration of sulfuric acid is raised above the proportions just described. In fact, more sulfuric acid was used in testing certain soil solutions.

EXPERIMENTAL RESULTS

COMPARATIVE LEVELS OF MICROBIAL ACTIVITY IN SOILS FROM FIELD PLOTS TREATED WITH SODIUM CHLORATE

The microbial activity on field plots treated September 15, 1939, with sodium chlorate at the rate of 200, 400, 600, and 800 pounds per acre have been compared. On one series the sodium chlorate was applied in solution, 1 pound of the salt per gallon of water, and on the other series the chlorate was applied in the dry form by means of a dry-chlorate spreader.

There were two series, duplicate plots per series, and two different dates of sampling for each series. The data may be combined inasmuch as the interest is not in the difference between wet and dry applications or between the dates of sampling. The primary purpose of the experiment was to determine the effect of the various rates of sodium chlorate applications on the level of microbial activity in the soil. This is expressed on the basis of the gas produced by the soil micro-organisms (see Materials and Methods) and is recorded in centimeters of mercury. Table 1 gives the results of the various treatments.

TABLE 1.—*Levels of microbial activity in soils treated with sodium chlorate at different rates on Sept. 15, 1939, at Lamberton, Minn., compared in terms of pressures, measured in centimeters of mercury, resulting from microbial activity in closed flasks.*

Treatment rate per acre (pounds)	Date of sampling and plot No.								Mean	Standard error of mean
	Sept. 9, 1940		Sept. 14, 1940		Sept. 19, 1940		Sept. 23, 1940			
	1	2	1	2	3	4	3	4		
	Cm.	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	
200	49.4	43.0	36.9	33.5	48.2	49.2	53.8	45.3	44.9	±1.67
400	29.4	44.1	31.2	38.8	51.6	45.2	54.2	47.2	42.7	±1.67
600	39.6	35.7	24.1	27.4	46.3	42.2	48.6	39.9	38.0	±1.67
800	24.4	31.6	19.8	25.7	45.6	37.4	29.3	33.0	30.8	±1.67

The F test of Snedecor (*16*) shows highly significant results for differences in treatments, indicating that the microflora of the soil was affected by the application of sodium chlorate at moderate and heavy rates. The 600- and 800-pound applications gave significantly lower values than the 200-pound application. The data on untreated areas were not included in the analysis of variance since check plots were not available within the blocks. However, by using the results which were obtained on untreated areas for comparison an interesting relationship is evident between microbial activity and the growth of annual weeds. This is shown in table 2.

TABLE 2.—*Levels of microbial activity and amount of vegetation on plots treated with sodium chlorate at different rates* [1]

Date of sampling	Plot No.	Microbial activity in plot series in which NaClO₃ was applied dry Sept. 15, 1939, at the rate per acre of—			
		200 pounds	400 pounds	600 pounds	800 pounds
1940		*Percent*	*Percent*	*Percent*	*Percent*
Sept. 9	1	107.0	49.0	76.8	48.1
Sept. 9	2	91.1	94.9	81.7	55.0
Sept. 14	1	95.1	91.4	45.0	41.3
Sept. 14	2	85.8	106.0	73.0	56.7
Average level of microbial activity		94.7	85.3	69.1	50.3
Estimated area covered by vegetation in September		90.0	80.0	60.0	40.0

[1] Values given are in percentages of values obtained for untreated areas (check=100 percent).

In table 2 it may be noticed that microbial activity decreased with a decrease in vegetation, which might have been expected. However, from the foregoing data it is impossible to differentiate between

the effect of the sodium chlorate and that of differences in amount of vegetation.

A similar study was made the following spring on a block of plots on which there was no vegetation. These plots were treated September 15, 1940, and sampled May 8, 1941. Consequently, regrowth of plants had not occurred after the application of sodium chlorate. The lack of vegetation can be explained both on the basis of the higher chlorate content likely to be present in the surface soil and the earliness of the season. In this experiment, duplicate plots were not used. However, plots close together were selected and duplicate samples were taken from each plot. The results are given in table 3.

TABLE 3.—*Comparative microbial activity in soils sampled May 8, 1941, on untreated areas and on areas treated with sodium chlorate at different rates on Sept. 15, 1940, Lamberton, Minn.*[1]

Treatment rate per acre (pounds)	Pressure in sample No.—		Average pressure
	1	2	
	Cm.	*Cm.*	*Cm.*
None	84. 5	81. 0	82. 7
200	89. 5	88. 0	88. 7
600	80. 5	73. 0	76. 7
800	82. 5	82. 5	82. 5

[1] The values are the sum of pressure readings in centimeters of mercury obtained while soils were incubated in closed flasks for 85 hours at 25° to 28° C.

The pressures resulting from microbial activity, given in table 3, suggest that sodium chlorate has little or no effect on microbial activity since the values obtained were high for all treatments. The values reported in table 3 are considerably higher than those in table 1. Numerous factors might have caused this difference, although it is usual for microbial activity to be at a high level during the spring season.

To study the probable effect of sodium chlorate on micro-organisms nearer to the time of application another series of plots was selected. These plots received treatments of sodium chlorate at the rate of 600 pounds per acre on the first of 3 successive months. An adjacent untreated area was studied for comparison with the treated area. A summary of the results obtained for the plots sampled on the various dates appears in table 4.

TABLE 4.—*Levels of microbial activity in soils from plots treated with sodium chlorate and from an immediately adjacent untreated area for various dates of sampling*

Date of sampling	Time of incubation	Temperature	Untreated grain-stubble area [1]	600 pounds NaClO₃ per acre applied [1]—		
				Aug. 1, 1941	July 1, 1941	June 1, 1941
1941	*Hours*	*°C.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
Aug. 14	89	28–29	49. 1	48. 9	42. 8	46. 7
Aug. 18	89	26–27	40. 2	42. 1	42. 5	
Aug. 29	89	32–34	136. 9	50. 5		
Sept. 4	84	29–30	93. 7	33. 5		

[1] Values given are the sums of pressure readings in centimeters of mercury.

In table 4 it is shown that the values obtained for samplings made during the middle of August are comparatively uniform throughout the various treatments. The samples taken August 29 and September 4 on two of the plots show that there was a marked difference between the treated and untreated areas. A comparison of the difference between the area treated August 1 and the untreated area for the two dates of sampling shows that the microbial activity on the untreated area was 270–280 percent above that on the treated area.

The microbial activity increased greatly in the early fall following the removal of the ripened grain crop. This may be explained by the fact that annual weeds became abundant in the untreated grain stubble during the latter part of August. As a result of this growth the surface layers of soil contained many roots, which undoubtedly favored the increase of at least certain species of micro-organisms. The sodium chlorate treatment inhibited the growth of annual weeds on the treated area. The application of chlorate apparently affected the supply of readily decomposable organic matter, which in turn limited the number of active organisms in the treated soil.

CHLORATE REDUCTION

Since the chlorate did not seem to have a direct detrimental effect on microbial activity, studies of chlorate reduction in the presence of a highly active flora were undertaken. The experiments were similar to those just described except that a uniform soil sample was used for each trial and chemical determinations of chlorate were made. There were also certain variations in treatment.

The soil samples for the following experiment were collected in the fall approximately 1 month after sodium chlorate applications on a grain-stubble field. The soils after incubation were left to dry at room temperature for 1 week on tin covers painted with asphalt paint. The treatment and results are given in table 5. A considerable amount of chlorate was reduced since 0.053 gm. of sodium chlorate can be taken as the amount present in all samples at the start. The reduction was not so marked in the samples treated with toluol.

TABLE 5.—*Representative data on 100-gm. portions of a composite sample of soil from a grain-stubble field previously treated with sodium chlorate, showing gas production and chlorate reduction under different conditions.*

Sample No.	Additions to soil	Pressure reading at indicated temperature and hours of incubation [1]—							Total [1]	KMnO₄ titration [2]	NaClO₃ per 100 gm. of air-dry soil
		32°C., 40.5 hours	32°C., 53 hours	33°C., 65 hours	34°C., 77 hours	32°C., 89 hours	32°C., 113 hours	32°C., 137 hours			
		Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cc.	Gm.
7	2 gm. dextrose +25 cc. H₂O	27	15.2	9.5	5.3	0.7	0.5	0	58.2	11.3	0.027
8	___do___	34	12.9	12.9	4.4	.7	.8	.2	65.9	12.0	.024
9	___do___	30	13.9	13.9	4.2	.7	.5	0	63.2	12.5	.031
10	___do___	34	13.8	8.5	4.7	.8	.5	0	62.3	11.9	.024
11	___do___	34	12.8	8.2	4.8	.4	1.1	0	61.3	8.9	.037
12	___do___	35	13.4	8.4	5.1	.8	.8	0	63.5	11.4	.027
19	2 gm. dextrose +25 cc. H₂O +1 cc. toluol	4.3	0	0	.7	−.4	0	0	4.6	8.1	.041
20	___do___	−.5	0	0	.5	−.5	0	.3	−.2	8.0	.041
21	___do___	10.3	1.2	0	.8	−.4	0	0	11.9	8.0	.041
29	None—dried immediately	-------	-------	-------	-------	-------	-------	-------	-------	5.3	.053

[1] Values given are in centimeters of mercury.

[2] Titration on water blank=17.6; titration for soil sample, without chlorate, following incubation=17.7.

A similar experiment was carried out on a different composite soil sample also taken from a stubble-field plot previously treated with sodium chlorate. In this trial less water was used and two of the samples were left unstoppered. The results, recorded in table 6, again show that a considerable amount of chlorate was reduced during the time of incubation or during the process of air-drying of the samples following incubation. Since 0.037 gm. of sodium chlorate was present at the start of the incubation period, it is apparent that nearly half of the chlorate was reduced.

TABLE 6.—*Reduction of chlorate in soil incubated 169 hours at 28°–30° C., and pressure readings on stoppered flasks*

Sample No.	Additions to soil	Condition of soil in Erlenmeyer flask	Sum of pressure readings	Sodium chlorate per 100 gm. air-dry soil
			Cm.	*Gm.*
21	2 gm. dextrose in 10 cc. water	Packed	46.1	0.020
22	....do	....do	39.8	.019
27	....do	....do	(¹)	.021
28	....do	....do	(¹)	.021
23	2 gm. dextrose in 20 cc. water	Unpacked	40.0	.021
24	....do	....do	74.7	.014
25	2 gm. dextrose in 20 cc. H₂O+1 cc. toluol	....do	.6	.031
26	....do	....do	4.4	.031
29	None—dried immediately without incubation			.037

¹ Erlenmeyer flask unstoppered.

Some variation was noted in the pressure readings and in the amount of chlorate reduced. Duplicate samples 23 and 24 were particularly irregular. Sample 24 showed the greatest amount of microbial activity and also the greatest amount of chlorate reduction. Increasing the water increased the average pressure readings, possibly because of the decrease in air volume or an increase in microbial activity resulting from the higher moisture content and better distribution of the dextrose. With relatively small additions of water (10 and 20 cc.), it was observed that the entire mass of soil may not become moistened immediately unless the soil is packed. Since the entire soil sample should be uniformly treated, all likelihood of the added material running down one side of the Erlenmeyer flask and coming in contact with only a portion of the soil should be eliminated. Packing the soil before adding the solution aided in its distribution and increased the uniformity of the results.

Experience has shown that in general heavier applications of sodium chlorate are required for weed control on fertile soils. As a basis for studying chlorate reduction in a very fertile soil a further experiment was undertaken. The soil selected was taken from a plot of land which had been used as a feeding lot for sheep during the fall of the year before sampling. No crop was grown during the following summer and weed growth was kept down by frequent cultivation. Since the soil had not been treated previously with sodium chlorate each individual 100-gm. sample was given 0.04 gm. NaClO₃ in solution at the time of beginning incubation. The previous samples tested for chlorate reduction were removed from the Erlenmeyer flasks and left to air-dry at room temperature for a week before the chlorate determinations were made. In this experiment the soils were not dried. The data reported in table 7 show a marked

TABLE 7.—*Gas production and chlorate reduction by a fertile soil supplemented with dextrose, sodium chlorate, and water just prior to incubation in closed vessels at 28–30° C.*

Sample No.	Additions to soil			Hours of incubation [1]													Sum of pressure readings	Titration KMnO3 [2]	NaClO3 remaining	Qualitative test
	Dextrose	NaClO3	Amount total solution	24	36	48	60	72	84	96	108	120	132	144	156	169				
	Gm.	*Gm.*	*Cc*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cc.*	*Gm.*	
1	2	0.04	10	21.2	28.0	22.9	20.3	17.7	17.2	14.7	8.5	6.4	6.4	5.8	6.5	8.7	184.3	17.9	0	Negative.
2	2	.04	10	21.6	28.2	24.0	23.0	21.8	21.7	20.8	13.6	10.5	9.9	7.6	7.9	10.4	221.0	17.8	0	Do.
3	2	.04	10	21.4	28.2	24.0	23.0	22.5	22.5	20.9	14.3	11.2	10.8	8.5	9.1	11.2	227.6	17.8	0	Do.
4	2	.04	20	35.0	33.0	29.4	27.4	23.4	17.4	10.0	5.1	4.5	6.0	6.5	8.1	9.3	215.1	19.9	0	Do.
5	2	.04	20	35.7	33.0	28.8	26.6	22.5	17.2	10.4	5.3	4.6	6.1	6.2	7.6	8.0	211.3	17.9	0	Do.
6	2	.04	20	11.6	10.5	10.2	20.8	28.7	26.1	19.8	11.5	9.4	9.3	8.2	8.0	9.5	183.6	17.7	0	Do.
7	0	.04	20	-.2	0	.8	.1	.9	.5	1.1	0	0	.8	.7	.6	1.1	6.4	15.2	.011	Positive.
8	0	.04	20	-1.6	0	.8	.2	1.0	.8	1.0	-.2	0	1.0	1.0	.8	1.4	6.2	17.2	.002	Do.

[1] Pressure values given are in centimeters of mercury.
[2] Titration on water blank = 17.7 cc.

acceleration in the rate of gas production and chlorate reduction as compared with the results reported in table 6.

MICROBIAL POPULATIONS IN SODIUM CHLORATE-TREATED SOILS UNDER CONTROLLED CONDITIONS

To determine the microbial populations in sodium chlorate-treated soils under controlled conditions by the dilution and plate-count methods a series of experiments was made.

Waukegan silt loam was used in the first experiment. The soil, after being air dried and sifted, was weighed into 100-gm. portions. Fifteen such samples were placed in glass tumblers and divided into three groups. The first group was left as a control, the second and third were treated with 0.01 and 0.035 gm. of sodium chlorate, respectively. Twenty-one cubic centimeters of water was added to each sample. The amount was determined by taking one-half the water-holding capacity of the soil. At intervals during the incubation period up to 2 months each group was sampled and microbial numbers determined. The results are given in table 8.

TABLE 8.—*Microbial counts at different times on Waukegan silt loam following treatment with sodium chlorate at different rates*

Time incubated	Number of organisms per gram of air-dry soil treated with—					
	No chlorate		0.01 percent of NaClO₃		0.035 percent of NaClO₃	
	Bacteria	Molds	Bacteria	Molds	Bacteria	Molds
	Millions	*Thousands*	*Millions*	*Thousands*	*Millions*	*Thousands*
2 days	57.5		58.2		50.2	
1 week	61.5		82.8		79.1	
2 weeks	44.1		43.2		46.0	
4 weeks	43.1	68	37.7	62	35.0	63
2 months [1]	26.7	55	40.0*	44	47.9*	48

[1] A determination of algae at this time showed no significant difference between treatments.
*Significantly different from no chlorate.

Since chlorate toxicity is usually considered more pronounced in poor than in fertile soils, a second experiment was conducted in which Anoka loess sand was used. Approximately the same concentrations of sodium chlorate were used expressed in terms of the dry weight of the soil. However, in terms of soil solution this was about double the concentration previously employed since only 10 cc. of water was added, which was one-half the water-holding capacity of the soil. The results are shown in table 9.

TABLE 9.—*Number of bacteria in a sandy soil at different times during a 2-month incubation period following treatment with different quantities of sodium chlorate*

Time incubated	Bacteria per gram of air-dry soil where chlorate added on basis of air-dry soil was—		
	None	0.01 percent	0.04 percent
	Millions	*Millions*	*Millions*
5 days	5.2	5.2	5.2
2 weeks	2.6	2.6	2.9
4 weeks	1.5	2.0*	3.3**
2 months	1.4	1.9*	2.9**

*Significantly different from no chlorate at 5-percent point; **significantly different from no chlorate at 1-percent point.

An examination of the data of table 9 shows that the bacteria survived better in the presence than in the absence of sodium chlorate under controlled conditions even though the chlorate concentration in terms of soil solution was high.

None of the plate counts thus far reported were made on soils in which chlorate had been present for a long time, or on soils to which organic matter was added. In table 9 are given bacterial counts and chlorate determinations for a soil, obtained at Lamberton, Minn., which had remained devoid of plant growth for more than 2 years as a result of treatment with sodium chlorate. The soil was air-dried, weighed into 100-gm. portions, placed in tumblers, treated, and incubated. The samples were air-dried again at the end of the incubation period, and quantitative analyses were made of the chlorate present. Because of previous experience with chlorate reduction in the presence of dextrose, 0.035 gm. of sodium chlorate was added to one group of tumblers along with the dextrose. If chlorate is reduced rapidly in the presence of a very active microflora, some chlorate would be expected to remain at the end of 2 weeks at this higher concentration.

In preparing the experiment it was assumed that the soil was relatively low in microbial activity. Upon adding dextrose an increase in the number of bacteria was anticipated. By adding dextrose it was possible to determine (1) whether bacteria would increase equally well in soils containing low and high concentrations of chlorate and (2) the extent of chlorate reduction at the different levels of microbial activity. The results are presented in table 10.

TABLE 10.—*Bacterial counts and chlorate determinations on soil samples from a field plot, which had remained devoid of vegetation for 2 years as a result of 1 sodium chlorate treatment, the samples having been given different treatments just prior to incubation.*

Incubation time at room temperature	Treatment just prior to incubation					
	None		1 gm. dextrose added per 100 gm. air-dry soil		1 gm. dextrose and 0.035 gm. sodium chlorate added per 100 gm. air-dry soil	
	Bacteria per gm. air-dry soil	NaClO₃ per 100 gm. soil	Bacteria per gm. air-dry soil	NaClO₃ per 100 gm. soil	Bacteria per gm. air-dry soil	NaClO₃ per 100 gm. soil
	Millions	*Gram*	*Millions*	*Gram*	*Millions*	*Gram*
None		0.015		0.015		0.050
3 days	53.2	.015		.014		.048
1 week			641	.013	579	.043
2 weeks	43.2	.015	425	.013	385	.042

The data in table 10 on bacterial counts are not extensive but the values obtained indicate that bacteria multiply in spite of the presence of chlorate. Accordingly, soils not supporting seed-producing plants because of chlorate treatment may be expected to increase in microbial activity when organic matter is added. Only a small part of the chlorate was reduced even though the bacterial activity was high in the samples supplemented with dextrose.

In connection with the experiment reported in table 10 a second group of tumblers was set up in which the fertile soil previously

referred to (p. 228) was used. In this group one-half of the tumblers were treated with 0.04 gm. of sodium chlorate. The results of this experiment are given in table 11.

TABLE 11.—*Bacterial counts on check and chlorate-treated portions of a very fertile soil, and chlorate determinations on treated samples for various periods of incubation*

Time incubated	Treatment		
	No NaClO₃	0.04 gm. NaClO₃ per 100 gm. air-dry soil	
	Bacteria	Bacteria	NaClO₃ remaining
	Millions	*Millions*	*Gram*
3 days	134	139	0.037
2 weeks	340	330	.034
6 weeks	35	32	.026
	32	35	.026

The data of table 11 show that the bacteria were not affected by the addition of sodium chlorate. The rate of chlorate reduction was not excessively rapid, though an appreciable amount of chlorate was reduced in a period of 6 weeks.

The soils treated with sodium chlorate and incubated at room temperature, with a moisture content equivalent to one-half the moisture-holding capacity of the soil, in no case showed a significant decrease in the number of micro-organisms. In certain instances there were significantly more bacteria in the chlorate-treated soils than in the untreated check samples (tables 8, 9). The chlorate was reduced slightly during the time of incubation under the conditions of the experiment. It was believed that a large number of micro-organisms in the soil would greatly hasten the reduction of sodium chlorate, but such was not the case in all instances. The discrepancy can be explained on the basis of the oxygen pressure in the soil. Further studies on the samples of soil analyzed for chlorate reduction and bacterial numbers reported in tables 10 and 11 suggest the factor of aeration.

A quantitative test of the chlorate remaining in the soil after not more than 2 weeks of incubation in tumblers at one-half the water-holding capacity of the soil showed that only a small amount of chlorate had been reduced. After the quantitative chlorate analyses were made the samples were left in the laboratory for 1 week submerged in water. Qualitative tests for chlorate were then made. The tests showed that during the 1 week in which the soils were submerged all the chlorate was reduced in the soils that had a high bacterial count, but not in those that had a low bacterial count. The samples that gave a positive test for chlorate were left submerged for as long as 2 months, but even then all the chlorate was not reduced. The soils showing a high degree of microbial activity evidently became highly reducing when the diffusion of oxygen into the soil became insufficient to supply the biological demand.

CROP YIELDS AND CHLORATE PERSISTENCE ON CHLORATE-TREATED PLOTS FERTILIZED WITH AMMONIUM SULPHATE, SODIUM NITRATE, AND MANURE

When chlorate is to be applied as an herbicide, it is important that enough be used to keep the soil toxic for a sufficient length of time to kill the perennial weeds. Upon eradication of the weeds, however, it is usually desired to resume cropping, and if applications of chlorate have been heavy efficient cropping is often delayed.

Since Crafts (*3*) had found that an increase in the mineral nutrient level of the soil was accompanied by a decrease in chlorate toxicity, a series of experiments was made in which ammonium sulphate, sodium nitrate, and manure were applied to chlorate-treated soil in field plots. The application of the commercial fertilizers was made at such rates that the nitrogen radical would be equal to, or a multiple of, the chlorate radicals applied. The plots were treated in September with sodium chlorate at the rate of 300 and 600 pounds per acre, and the following June ammonium sulphate, sodium nitrate, and manure were applied. The plots were then plowed and seeded to soybeans (*Soja max*). The soybeans, a crop relatively sensitive to chlorate, showed chlorate injury in various degrees throughout the summer. The chlorate injury was reflected in reduced yields of forage (table 12). The lowest yields were obtained from plots in which apparent chlorate injury was most severe.

TABLE 12.—*Sodium chlorate present in the soil and soybean yields (forage), as obtained from samples taken on sodium chlorate-fertilizer plots at the Lamberton Weed Experiment Station, Sept. 26, 1941*

Plot No.	Treatment (per acre)		Soybean yield per acre	Sample depth	NaClO$_3$ in air-dry soil
	NaClO$_3$ applied Sept. 16 1940	Fertilizer applied June 6 1941			
	Pounds		*Tons*	*Inches*	*P. p. m.*
L-1	None	None	2.12		
L-2	600	480 pounds NaNO$_3$	.66	12-24 / 24-36 / 36-48	13 / 39 / 17
L-3	600	374 pounds (NH$_4$)$_2$SO$_4$	.67	12-24 / 24-36 / 36-48	4 / 17 / 13
L-4	600	40 tons manure	1.52	12-24 / 24-36 / 36-48	0 / 0 / 4
L-5	None	374 pounds (NH$_4$)$_2$SO$_4$	2.16		
L-6	600	None	.56	12-24 / 24-36 / 36-48	0 / 30 / 12
L-22	None	do	1.69		
L-23	300	240 pounds NaNO$_3$	.85	12-24 / 24-36 / 36-48	4 / 21 / 17
L-24	300	187 pounds (NH$_4$)$_2$SO$_4$	.76	12-24 / 24-36 / 36-48	4 / 13 / 13
L-25	300	20 tons manure	1.44	12-24 / 24-36 / 36-48	0 / 4 / 13
L-26	None	374 pounds (NH$_4$)$_2$SO$_4$	1.96		
L-27	300	None	.97	12-24 / 24-36 / 36-48	4 / 13 / 21
L-28	300	480 pounds NaNO$_3$	.75	12-24 / 24-36 / 36-48	13 / 30 / 21

Contrary to expectation, chlorate injury did not decrease with an increase in the applications of commercial nitrogen fertilizers. Because of the apparent failure of the nitrogen to decrease the chlorate toxicity, samples were taken from the respective plots to determine whether there was any relation between the residual chlorate in the soil and the crop yield. The soil samples taken to a depth of 4 feet showed that the crop yields were lowest where the larger amounts of residual chlorate remained. Apparently chlorate reduction was greatest where manure had been applied. This observation is believed to support the previous evidence of chlorate reduction in the presence of a highly active soil microflora. Although the amount of chlorate remaining in the soil at the time of sampling does not necessarily correlate very closely with yield, it is interesting to note that yield was highest and residual chlorate was lowest on the chlorate treated plots to which manure was applied. No chlorate was found in the surface foot of soil on any of the plots.

The data of table 12, together with the other results, serve to show that the amount of chlorate applied is only one of a number of factors that may influence the quantity of chlorate remaining in the soil.

DISCUSSION

By merely recording the pressures produced over a definite time of incubation the relative microbial activity of soils can be compared. The pressures were considerably reduced by the addition of toluol, thus supporting the view that microbial activity was being measured. The amount of gas produced appeared to correlate well with the numbers of soil micro-organisms and fertility. Good results were obtained when the soils were taken directly from the fields, prepared, and incubated without any loss of time between sampling and the beginning of incubation.

The duration of gas production was relatively short and differed somewhat between soils. It was apparent that the accumulation of staling substances was rapid in all soils, as a foul odor was produced. Gillespie (5) reports that accompanying the development of reducing conditions there is the production of a foul odor in most cases, and that some soils become highly reducing when waterlogged.

By analyzing all the data available it appears that organic matter does play a role in the reduction of chlorate as it is a source of energy for the micro-organisms which apparently are an important factor in causing rapid chlorate reduction in soils. During the process of rapid multiplication the increasing numbers and activities of the normal microbes of the soil greatly increase the oxygen demand. Warm temperatures and moist conditions favor microbial growth. On the other hand, increasing the soil moisture decreases the air volume and rate of diffusion into the soil. A consequence of the two actions is an oxygen deficit due to an increased oxygen demand and decreased supply. This results in an accumulation of reducing substances and probably a lowering of the oxidation-reduction potential of the soil. Whether the chlorate reacts with the reducing substances under the conditions of low oxygen pressure, or whether its reduction is the result of a direct biological action, cannot be ascertained from these results. The data do indicate that the normal micro-organisms of the soil are important in creating an oxygen deficit under certain conditions and then directly or indirectly cause a rapid reduction of chlorate.

Whether it be specific organisms or the medium, the influence of the total flora is of primary importance since the flora in general establishes the oxygen demand.

The rate of chlorate reduction seemed to be directly proportional to the rate of formation of reducing substances. The presence of these reducing substances became apparent when the samples were being titrated with permanganate. Several additional cubic centimeters of permanganate solution could be added after the proper end point had been observed and the color would disappear in a short time. The error resulting from reducing substances present in the soil following the clearing of the soil solution as best one could was kept at a minimum by taking the value at which the sample being titrated was first completely colored pink during vigorous shaking. The determination of this point is largely dependent on the clearness of the sample aliquot being titrated.

The addition of Zimmermann-Reinhardt solution was heplful in clearing the sample before titrating with standard potassium permanganate and in increasing the accuracy of the results. When practical a qualitative test was used to confirm the quantitative test. The quantitative method was further checked by comparison with Rosenfels' (*13*) sulfurous acid reduction method.

For rapid chlorate reduction, in addition to high microbial activity there must be incomplete soil aeration, which occurs with heavy precipitation, packing of the soil, and poor drainage. Chlorate was reduced nearly as fast when the soil was packed and the Erlenmeyer flasks left unstoppered as when the flasks were stoppered (table 6).

A year of work in one locality showed that increasing the nitrate content of the soil by applications of commercial fertilizer did not reduce chlorate toxicity under field conditions, while the application of manure resulted in higher yields of soybeans and some reduction of chlorate. Since chlorate has been found to concentrate in plants by movement with the transpiration stream it is not unlikely that under conditions of rapid transpiration the action of nitrates would become subordinate.

In view of the results reported in this paper the rate of chlorate reduction is probably a major factor in determining the efficiency of sodium chlorate as an herbicide. It is likely that persistent plants eventually will be killed by the continued absorption of chlorate even though the rate of absorption is slow. The soybean plants grown on chlorate-treated plots showed more injury from chlorate as the plants developed despite a decrease in the amount of chlorate in the soil with time.

SUMMARY AND CONCLUSIONS

Studies were made of microbial populations and microbial activity in soils treated with sodium chlorate in the amounts usually employed in weed control. Rates of sodium chlorate reduction were observed also. Trials were conducted in the laboratory and in the field. The following conclusions were reached:

(1) Sodium chlorate applied in moderate concentrations does not exert a direct detrimental effect on the soil microflora.

(2) Sodium chlorate applications sterilized the soil for higher plants only and did not interfere with the growth of common soil organisms.

(3) Heterotrophic organisms are limited in their development on treated soil by a decrease in organic matter due to a lack of higher plant growth.

(4) The addition of organic matter to the soil immediately stimulates microbial growth in the presence as well as in the absence of sodium chlorate.

(5) Sodium chlorate is reduced in the presence of active soil microbes. The micro-organisms of the soil are directly or indirectly responsible for the rapid reduction of chlorate.

(6) The rate of chlorate reduction increases with microbial activity under conditions of poor aeration. Packing and moistening of soils which have very active microflora increase the rate of chlorate reduction.

(7) Chlorate is reduced rapidly in a fertile soil when the soil is waterlogged at warm temperatures.

(8) Reducing substances caused by microbial activity under conditions of low oxygen pressure are associated with the disappearance of chlorate in soils.

(9) Aerated soils supplied with readily available organic matter will reduce sodium chlorate in amounts equivalent to 800 pounds per acre when submerged in water, or flooded, for a few days.

(10) Sudden changes in microbial activity and chlorate content of soils are possible. The conditions under which chlorate is applied greatly influence the results.

The reason for the greater success with fall applications of sodium chlorate, and the generally poor results on highly fertile soils under moist warm conditions, can be explained on the basis of these experimental results.

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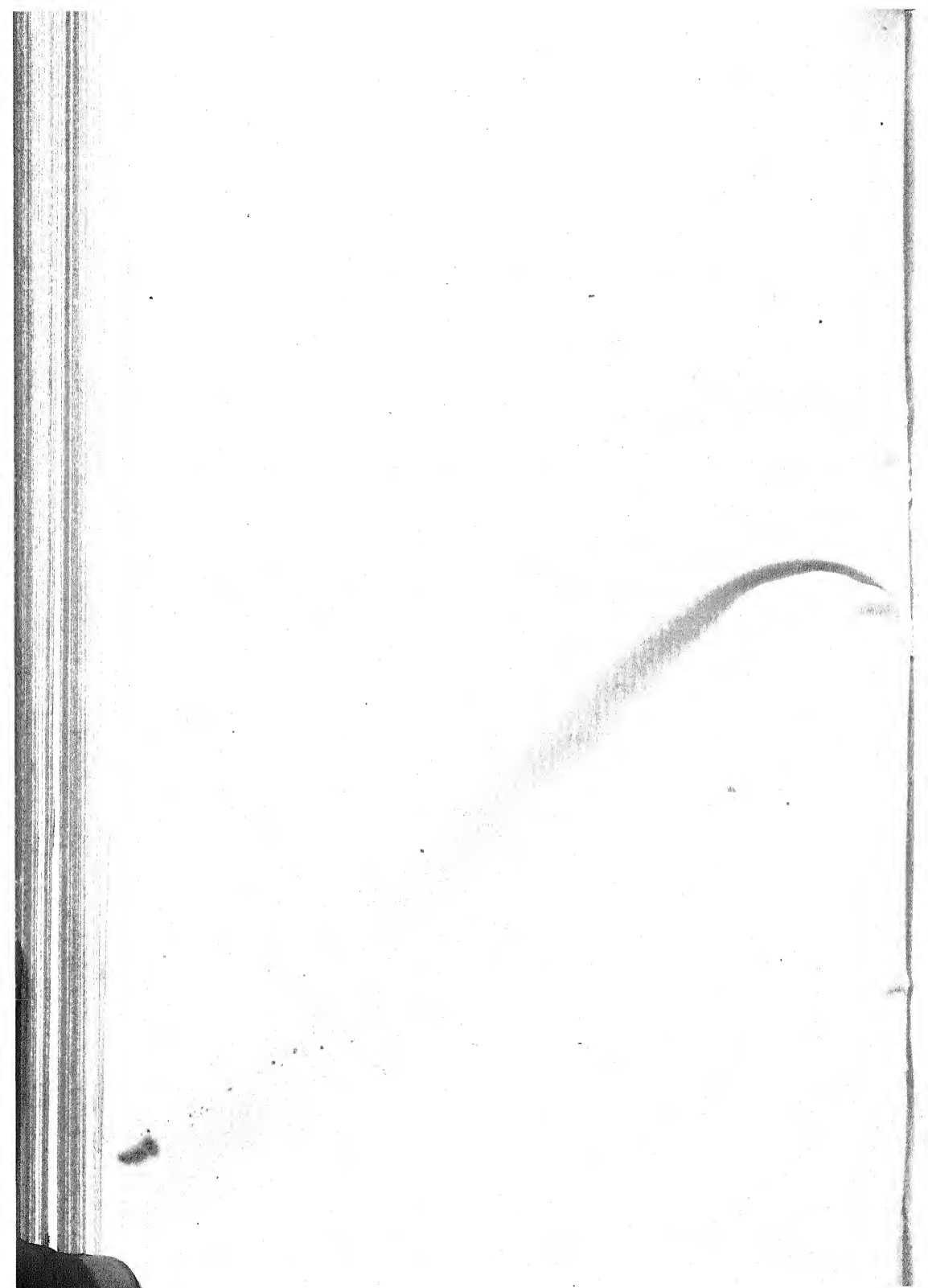
METHODS OF ESTIMATING THE PHYSICAL AND CHEMICAL COMPOSITION OF CATTLE[1]

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INTRODUCTION

For certain practical purposes the relative fatness of cattle is commonly expressed as a specified 'degree of "condition" or "finish." Such personal judgment serves a need in marketing, but for research purposes fatness is best measured by knowledge of the chemical and physical composition of the live animal and of the carcass. Detailed analyses are laborious, costly, and often impractical. The purpose of this paper is to show that the composition of the entire animal and of the carcass can be predicted reliably from the analysis of only a small portion of the carcass.

REVIEW OF LITERATURE

Murray (9)[4] observed that the bodies of farm animals are composed of fat and nonfatty matter which varies within wide limits, depending on the feeding. For the animals studied, he noted that the percentage of chemical composition of the fat-free matter—moisture, ash, and protein—is practically the same and is not affected by the condition or the degree of fatness of the animal and to only a small extent by age.

Moulton (4) showed that the composition of animals should be compared on the fat-free basis and that the fatness of the individual has no effect on the composition calculated on the fat-free basis. Edema, underdevelopment, and atrophy were observed to give abnormal water percentages. He found that mammals reach chemical maturity at different ages and that these ages are a fairly constant part of the total life cycle. For cattle he observed that chemical maturity is reached at about 5 months after birth, after which time the chemical composition of the animal on the fat-free basis is a practical constant, showing but little decrease in water and but little increase in ash and protein percentages with advancing age.

Murray (9) stated that the chemical composition of the live animal may be estimated if the fatness is known, assuming a constant composition for the fat-free live animal body. Moulton, Trowbridge and

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Haigh (*6*) when at the University of Missouri observed that for cattle the wholesale rib cut was representative of the carcass. Lush (*3*) studied various practical relations for estimating the proportion of fat and bone in cattle, using the published data of the Missouri, Illinois, Maine, and Rothamsted stations, and concluded that the percentage of fat in the edible portion (lean and fat flesh) of the wholesale rib cut is the more accurate indicator of the degree of fatness of the entire live animal.

Chatfield (*1*) found that "for any given wholesale cut there is a close relation between the content of fat determined as ether extract and that of the visible fat." Lush (*3*) found the relation between the ether extract in the edible portion of the wholesale rib cut and the ether extract in the entire live animal to be linear and to have a very high coefficient of correlation (+0.987). Lush noted that differences in sex and breed of the cattle slaughtered and differences in slaughter technique seemed to have less influence upon the wholesale rib cut as an indicator than upon the other indicators he studied.

In addition to the composition on the fat-free basis being a function of age in immature animals (*4*), it may be deducted that it is also a function of fatness in mature beef animals as Chatfield (*1*) concluded that the protein content of the edible portion of fresh mature beef sides is a curvilinear function of the fat content.

Lush (*3*) studied the relations referred to the entire live animal. The literature indicates, and recent results obtained at the North Dakota station show, that there is a large difference in the digestive fill or contents of the stomachs, intestines, and bladder, calculated as a percentage of the pasture or feed-lot live weight, influenced by the character of the ration consumed and possibly by the fatness of the animal. Sufficient data relative to these factors are not available to permit an estimate of the digestive fill or contents from knowledge of the ration consumed, but such data may be anticipated. Hence it appears that in working out indicator relations for the live animal they should be calculated to indicate the fatness of the warm empty body, that is, on the basis of the pasture or feed-lot live weight less the determined or estimated digestive fill or contents. The warm or "hot" carcass weight should also be used inasmuch as the chilled carcass weight is an empirical value depending on the washing loss and the losses of storage influenced by fatness and length of time of aging.

It has recently been suggested that the ninth, tenth, and eleventh rib portion of the wholesale rib cut is a more satisfactory or representative portion of the empty body and warm carcass than the wholesale rib cut. Hereafter in this presentation the ninth, tenth, and eleventh rib cut will be referred to as the 9–11 rib cut.

A study of the relations between the physical and chemical composition of the empty body, warm carcass, and edible portion of the warm carcass, and the physical and chemical composition of the whole and edible portions (lean and fat flesh) of the wholesale and 9–11 rub cuts was made.

THE BASIC DATA

It is obvious that the data avaiable for the study would be restricted to those experiments in which cattle were carefully slaughtered and the entire animal and certain parts fully accounted for in the physical and chemical analyses. Two groups of analyses were

found available.   They were the published analyses of 33 steers and 3 cows from Missouri Agricultural Experiment Station bulletins (*5, 6, 7, 8, 10,* and *11*) and the unpublished analyses of 56 steers from the files of the North Dakota Agricultural Experiment Station.   The slaughter record, carcass and wholesale rib-cut separation into lean, fat, and bone, and chemical analysis of empty body, carcass, and wholesale rib cut were found complete for 36 Missouri animals (28 steers from the "Use of Food" experiments, 5 steers from the "Retarded Growth" experiments, and 3 Jersey cows).   For the North Dakota steers the available data included slaughter record, carcass, wholesale rib, and 9–11 rib separation into lean, fat, and bone, and chemical analysis of the 9–11 rib cut.   The 9–11 rib cuts were further separated into eye muscle, other lean, fat, and bone for chemical analysis.   The eye muscle is the longissimus dorsi taken without the superficial fascia or muscle lying outside the connective tissue. It represents clear lean or lean with only the fat of marbling.

The data for the Missouri animals were calculated to give the physical and chemical composition of the empty body, carcass, edible portion of carcass, wholesale rib cut, and edible portion of the wholesale rib cut.   It was found necessary to calculate some of the percentages from the data in the original publications and to recalculate some others.   In the calculations slaughter losses and shrinkages were considered as moisture and were so caluclated in recording the chemical composition and distributed proportionally between lean, fat, and bone in recording the physical composition.

The data for the North Dakota steers were calculated to give the physical composition of the empty body, whole and edible portions of the carcass, wholesale rib cut, and 9–11 rib cut, and the chemical composition of the whole and edible portion of the 9–11 rib cut.

In the tabulations and discussion of the date the term "fat" is used to represent the separable fatty adipose tissue.   The term "ether extract" is used to represent the true chemical fat.   For practical purposes it is assumed that moisture, ash, crude protein ($N \times 6.25$), and ether extract represent the total composition of the animal body and its several parts.   In the physical composition of the empty body the lean included that of the carcass, head, and tail; the fat that of the carcass, head, and offal (stomachs and intestines); the bone that of the carcass, head, tail, and feet; and the offal the rest of the animal body.

The basic data giving the age, weights, dressing percentages, and the physical and chemical composition are not presented in full detail.   A summary of the data on the age, weights, dressing percentages, and physical composition is given in table 1, and of the chemical composition in table 2.   These summary tables give the lowest and highest values, the range, the standard deviation, and the mean value for each of the various items of the basic data.

TABLE 1.—*Summary of data on age, weights, dressing percentages, and physical composition of the animals*

Item	Missouri animals (36)					North Dakota animals (56)					All animals (92)				
	Lowest value	Highest value	Range	Standard deviation (±)	Mean	Lowest value	Highest value	Range	Standard deviation (±)	Mean	Lowest value	Highest value	Range	Standard deviation (±)	Mean
Slaughter data:															
Age ___months	3	112	109	24.34	28.36	13	35	22	6.28	26.14	3	112	109	15.90	27.01
Live weight[1] ___pounds	187	1,948	1,761	688.60	799.37	470	1,325	855	231.85	956.22	187	1,948	1,761	360.88	894.84
Empty-body weight_do	157	1,796	1,639	450.69	715.22	347	1,178	831	219.48	766.38	157	1,796	1,639	329.18	746.37
Warm-carcass weight ___do	100	1,343	1,243	321.82	479.94	210	800	590	158.64	501.31	100	1,343	1,243	240.26	492.95
Digestive fill as percentage of live weight ___percent	6.74	17.72	10.98	2.76	11.60	11.08	31.56	20.48	5.59	20.71	6.74	31.56	24.82	6.46	17.14
Dressing percentage:															
Live-weight basis ___do	53.00	70.42	17.42	4.55	57.75	43.11	61.15	18.04	4.92	51.51	43.11	70.42	17.31	5.65	53.95
Empty-body basis_do	58.82	75.52	16.70	3.75	65.27	60.52	69.62	9.00	2.30	64.97	58.82	75.52	16.70	2.93	65.08
Physical composition:															
Empty body:															
Lean ___do	31.92	48.10	16.18	3.86	41.94	35.99	46.80	10.81	2.59	41.09	31.92	48.10	16.18	3.10	41.42
Fat ___do	.73	40.13	39.40	10.04	14.17	3.06	29.43	26.37	6.91	14.28	.73	40.13	39.40	3.22	14.23
Bone ___do	9.38	26.47	17.09	4.34	16.84	11.08	19.84	8.72	2.37	15.59	9.38	26.47	17.09	3.32	16.08
Offal ___do	17.96	32.24	14.28	3.58	27.05	23.11	35.96	12.85	3.15	29.04	17.96	35.96	18.00	3.44	28.26
Carcass:															
Lean ___do	42.22	71.85	29.63	7.22	63.81	53.49	73.68	20.19	5.22	64.56	42.22	73.68	31.46	5.97	64.26
Fat ___do	.16	47.52	47.36	11.67	16.37	3.40	34.40	31.00	8.27	17.42	.16	47.52	47.36	9.69	17.01
Bone ___do	9.99	34.10	24.11	5.52	19.82	12.11	24.50	12.39	3.29	18.02	9.99	34.10	24.11	4.36	18.72
Edible portion of carcass:															
Lean ___percent	47.05	99.76	52.71	12.84	80.35	60.86	95.59	34.73	9.32	79.10	47.05	99.76	52.71	10.78	79.59
Fat ___do	.24	52.96	52.71	12.84	19.65	4.41	39.14	34.73	9.32	20.90	.24	52.95	52.71	10.78	20.41
Wholesale rib cut:															
Lean ___do	37.79	71.93	34.14	8.01	63.26	50.69	72.66	21.97	5.55	63.07	37.79	72.66	34.87	6.57	63.14
Fat ___do	2.18	50.76	50.76	13.14	12.62	2.18	37.23	35.05	9.02	16.32	2.18	50.76	50.76	11.90	14.87
Bone ___do	11.45	41.65	30.20	6.96	24.13	12.08	29.29	17.21	3.94	20.61	11.45	41.65	30.20	5.57	21.99
Edible portion of wholesale rib cut:															
Lean ___percent	42.68	100.00	57.32	15.01	84.53	57.65	96.99	39.34	10.41	79.91	42.68	100.00	57.32	12.54	81.72
Fat ___do	0	57.32	57.32	15.01	15.47	3.01	42.35	39.34	10.41	20.09	0	57.32	57.32	12.54	18.28
9-11 rib cut:															
Lean ___do						47.84	71.30	23.46	6.12	60.92					
Fat ___do						2.92	41.37	38.45	9.96	18.52					
Bone ___do						10.79	30.03	19.24	4.38	20.56					
Edible portion of 9-11 rib cut:															
Lean ___percent						53.62	95.97	42.35	11.43	77.25					
Fat ___do						4.03	46.38	42.35	11.43	22.75					

[1] Pasture or feed-lot live weight.

TABLE 2.—*Summary of data on the moisture, ash, crude protein, and ether extract content of the animals expressed on a percentage basis*

Animals and part analyzed	Moisture					Ash					Crude protein					Ether extract				
	Lowest value	Highest value	Range	Standard deviation (±)	Mean	Lowest value	Highest value	Range	Standard deviation (±)	Mean	Lowest value	Highest value	Range	Standard deviation (±)	Mean	Lowest value	Highest value	Range	Standard deviation (±)	Mean
Missouri animals (36):																				
Empty body	29.83	71.52	31.69	8.02	58.70	2.97	7.00	4.03	0.80	4.62	12.27	20.59	8.32	2.27	17.95	2.16	44.56	42.40	10.68	18.14
Carcass	36.77	71.72	34.95	8.89	57.04	3.03	8.26	5.23	1.01	5.16	10.22	19.92	9.70	2.46	17.35	2.20	49.83	47.63	11.96	20.45
Edible carcass	37.20	77.25	40.15	10.82	62.10	.48	1.16	.68	.16	.86	9.00	19.98	10.98	2.81	16.93	1.89	53.32	51.43	13.71	20.11
Wholesale rib	31.22	71.74	40.52	9.87	55.74	3.57	9.92	6.35	1.36	6.34	10.13	21.32	11.19	2.86	17.78	2.41	55.08	52.67	13.40	20.12
Edible wholesale rib	31.03	77.28	46.25	12.34	61.98	.41	1.16	.75	.19	.86	8.74	20.71	11.97	3.21	17.09	2.09	59.82	57.73	15.66	20.08
North Dakota animals (56):																				
9–11 rib	37.97	67.29	29.32	7.83	53.39	4.39	9.08	4.69	1.16	6.70	13.09	22.72	9.63	2.31	18.46	2.68	44.55	41.87	10.91	21.45
Edible 9–11 rib	39.18	76.45	37.27	9.97	58.81	.57	1.09	.52	.13	.84	11.67	21.91	10.24	2.51	17.22	1.84	48.58	46.74	10.57	23.13
Fat of 9–11 rib	8.34	67.72	59.38	15.73	22.82	.15	1.02	.87	.22	.38	2.65	15.78	13.13	3.57	7.35	16.18	88.86	72.68	19.32	69.75
Bone of 9–11 rib	23.76	46.52	22.76	5.08	33.18	22.69	36.59	13.90	3.31	29.72	21.19	25.90	4.71	.89	23.62	4.13	20.26	16.13	3.55	12.81
Eye muscle, 9–11 rib	70.40	78.28	7.88	1.86	74.56	1.00	1.16	.16	.03	1.06	20.44	22.99	2.55	.47	21.64	.39	6.74	6.35	1.53	2.60

DISCUSSION OF ANIMALS AND METHODS

The data available for study gave a wide variation in the breeding, age, weight, and fatness of the animals. The ages ranged from 3 to 112 months at the time of slaughter, the empty-body weight from 157 to 1,796 pounds, and ether extract in the empty body from 2.2 to 44.6 percent. Aside from the 3 aged Jersey cows, the animals were of the beef-type breeds and their crosses. The 33 Missouri steers included purebred Herefords, and Shorthorns, and Hereford-Shorthorn crosses. Their ages ranged from 3 to 48 months. The ages of the cows were 76, 85, and 112 months respectively. The 56 North Dakota steers included animals from 4 groups used in the range-grazing and lot-feeding experiments. They were range-reared Herefords of apparently good breeding and ranged from 13 to 35 months in age. Two of these groups of steers were obtained from western North Dakota ranches, one from Canada, and the fourth was purchased on the feeder market at Kansas City, Mo.

The advisability of using the empty-body weight instead of the feed-lot, pasture, or slaughter live weight is evident from the variations in the percentage of digestive fill (table 1), which are certainly influenced by the character of the ration and possibly by the weight and fatness of the animal. The digestive fill is thought to be best expressed as a percentage of the feed-lot or pasture live weight. The variation is striking in the case of the North Dakota steers. The percentages for the steers grazing on the native short-grass pasture averaged 25.2, 24.1, 19.1, and 27.5 for the grazing seasons of 1933, 1934, 1935, and 1936 respectively. The low average value for 1935 may be accounted for by the fact that the pasturage available was all new growth and of good quality. The range pasture had been grazed bare the previous season. The high value for 1936 may have resulted from the poor quality and fibrous nature of the pasturage carried over from 1935. As a result of drought there was practically no growth of vegetation in 1936. In the feed lot on grain-fattening rations steers from the same groups averaged 14.0, 14.4, 13.7, and 17.3 percent of digestive fill respectively. The higher average value of 17.3 for the 1936 group was perhaps due to the feeding of light-weight barley, high in crude fiber. For the animals studied the digestive fill ranged from 6.74 to 31.56 percent of the pasture or feed-lot live weight.

The data on the physical and chemical composition have been used as a basis for the calculation by the method of least squares of linear equations which might be of use in estimating the composition of the empty body, carcass, and edible carcass from the rib cuts. The statistical formulae (2, 12) used were:

$$\text{Standard deviation} = \sigma_x = \sqrt{\frac{\Sigma(x^2) - \frac{(\Sigma x)^2}{n}}{n-1}}$$

$$\text{Standard error of mean} = \frac{\sigma_x}{\sqrt{n}}$$

$$\text{Correlation coefficient} = r_{xy} = \frac{\Sigma(xy) - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{\left[\Sigma(x^2) - \frac{(\Sigma x)^2}{n}\right]\left[\Sigma(y^2) - \frac{(\Sigma y)^2}{n}\right]}}$$

Standard error of estimate $= S_{yx} = \sqrt{\dfrac{\Sigma(y^2) - \dfrac{(\Sigma y)^2}{n}}{n-2}(1-r^2_{xy})}$

Partial correlation coefficient $= r_{xa \cdot b} = \dfrac{r_{xa} - (r_{xb})(r_{ab})}{\sqrt{(1-r^2_{xb})}\sqrt{(1-r^2_{ab})}}$

The probable error of estimate was not calculated. If desired, it may be considered as being two-thirds of the standard error of estimate. The formulae are given in expanded forms which when used for machine calculation introduce the least mechanical error.

As is evident, some of the correlation coefficients are of a part correlated with the whole. This does not invalidate the use of the regression equations for prediction, but does invalidate the usual tests of significance whose purpose is to detect small differences between correlation coefficients derived from such data. However, if a small part shows a correlation with the whole of 0.98, for example, and another such correlation has a value of 0.96, both should be considered about equally reliable. Further when one such value is 0.98 and another is 0.75 it seems likely that the difference is a real one if the series are fairly large. In this paper emphasis is given to the standard errors of estimate as a measure of the usefulness of the regression equations for prediction purposes. As a consequence, correlation coefficients are used but sparingly in judging the value of the regression relations, but are included in the tables for the interest of the general reader.

RELATIONS IN PHYSICAL COMPOSITION

The relations calculated between the percentages of lean, fat, and bone in the rib cuts and the percentages of lean, fat, and bone in the empty body, carcass, and the edible portion of the carcass were found to have high positive correlation coefficients and the regressions appear to be linear (table 3).

The percentage fat relations, in which most interest is centered, have particularly high coefficients, +0.97 or higher, and relatively low standard errors of estimate and should be satisfactory for prediction purposes.

The relations for the estimation of the lean in the empty body have lower correlation coefficients than those for the estimation of the fat but the standard errors of estimate are relatively low considering the percentage of lean in the empty body. It must be noted that practically all of the lean in the empty body is accounted for in the carcass portion of the body and that there is considerable variability in the percentage of the offal and its parts, such as the head, feet, and hide. This variability may be illustrated by citing an observation of the variation in the hide weight from 82 to 132 pounds (average 101.6 pounds) per steer for 30 Hereford steers averaging 1,135 pounds slaughter live eight and slaughtered on the same date.

The relations for the estimation of the lean in the carcass and edible portion of the carcass (table 3) have correlation coefficients of high order, ranging from +0.88 to +0.98. The 9–11 rib cut appears to be the better indicator on the basis of standard errors of estimate. For the edible portion of the carcass the edible portion of the rib cuts

TABLE 3.—*Relations in Physical Constituents*

Relation No.	X	Y	n	r_{xy}	Regression of Y on X	
					Equation	S_{yx} (±)
1	Lean, wholesale rib	Lean, empty body	92	0.841	$Y=0.40422 X+15.89934$	1.714
2	Lean, edible wholesale rib	do	92	.804	$Y= .20264 X+24.86366$	1.885
3	Lean, 9-11 rib	do	56	.715	$Y= .30270 X+22.65161$	1.827
4	Lean, edible 9-11 rib	do	56	.728	$Y= .16522 X+28.32942$	1.791
5	Fat, wholesale rib	Fat, empty body	92	.973	$Y= .73396 X+ 3.31928$	1.900
6	Fat, edible wholesale rib	do	92	.970	$Y= .63594 X+ 2.60774$	2.006
7	Fat, 9-11 rib	do	56	.985	$Y= .68371 X+ 1.61401$	1.180
8	Fat, edible 9-11 rib	do	56	.981	$Y= .59276 X+ .79290$	1.345
9	Bone, wholesale rib	Bone, empty body	92	.965	$Y= .57509 X+ 3.43707$	.874
10	Bone, 9-11 rib	do	56	.947	$Y= .51327 X+ 5.04322$	.764
11	Lean, wholesale rib	Lean, carcass	92	.958	$Y= .88148 X+ 8.60471$	1.745
12	Lean, edible wholesale rib	do	92	.929	$Y= .44873 X+27.59468$	2.241
13	Lean, 9-11 rib	do	56	.940	$Y= .80173 X+15.71220$	1.784
14	Lean, edible 9-11 rib	do	56	.960	$Y= .43838 X+30.69027$	1.470
15	Fat, wholesale rib	Fat, carcass	92	.981	$Y= .87296 X+ 4.02921$	1.857
16	Fat, edible wholesale rib	do	92	.980	$Y= .75784 X+ 3.15617$	1.920
17	Fat, 9-11 rib	do	56	.984	$Y= .81774 X+ 2.27664$	1.459
18	Fat, edible 9-11 rib	do	56	.982	$Y= .71026 X+ 1.26501$	1.574
19	Bone, wholesale rib	Bone, carcass	92	.974	$Y= .76344 X+ 1.94001$	.983
20	Bone, 9-11 rib	do	56	.941	$Y= .70750 X+ 3.47863$	1.120
21	Lean, wholesale rib	Lean, edible carcass	92	.880	$Y=1.44356 X-11.56371$	5.142
22	Lean, edible wholesale rib	do	92	.979	$Y= .84248 X+10.74122$	2.185
23	Lean, 9-11 rib	do	56	.940	$Y=1.43280 X- 8.19347$	3.185
24	Lean, edible 9-11 rib	do	56	.982	$Y= .80161 X+17.17102$	1.748
25	Fat, wholesale rib	Fat, edible carcass	92	.978	$Y= .96820 X+ 6.01490$	2.239
26	Fat, edible wholesale rib	do	92	.979	$Y= .84248 X+ 5.01078$	2.185
27	Fat, 9-11 rib	do	56	.983	$Y= .92088 X+ 3.84738$	1.727
28	Fat, edible 9-11 rib	do	56	.982	$Y= .80161 X+ 2.66798$	1.748
29	Lean, edible wholesale rib	Lean, wholesale rib	92	.908	$Y= .47676 X+24.18313$	2.759
30	Lean, 9-11 rib	do	56	.967	$Y= .87618 X+ 9.69149$	1.422
31	Lean, edible 9-11 rib	do	56	.949	$Y= .46068 X+27.48255$	1.762
32	Lean, 9-11 rib	Lean, edible wholesale rib	56	.958	$Y=1.62919 X-19.34614$	2.997
33	Lean, edible 9-11 rib	do	56	.988	$Y= .90004 X+10.37911$	1.601
34	do	Lean, 9-11 rib	56	.970	$Y= .51978 X+20.76896$	1.498
35	Fat, edible wholesale rib	Fat, wholesale rib	92	.998	$Y= .86778 X- .99375$	2.010
36	Fat, 9-11 rib	do	56	.989	$Y= .89609 X- .27648$	1.302
37	Fat, edible 9-11 rib	do	56	.985	$Y= .77710 X- 1.35747$	1.538
38	Fat, 9-11 rib	Fat, edible wholesale rib	56	.988	$Y=1.03403 X+ .93973$	1.570
39	Fat, edible 9-11 rib	do	56	.988	$Y= .90004 X- .38311$	1.601
40	do	Fat, 9-11 rib	56	.997	$Y= .86890 X- 1.24474$	.667
41	Bone, 9-11 rib	Bone, wholesale rib	56	.974	$Y= .87830 X+ 2.55467$	.894

should be a more reliable indicator than the whole rib cuts as the relations have lower standard errors of estimate.

As indicators of fatness the whole and edible portions of the wholesale and 9–11 rib cuts all appear to be quite reliable (table 3). The regression coefficients are all less than one, showing a greater fatness in the rib cuts than in the gross portions. The range in the Y intercepts of the relations for each of the gross portions is not excessive, and the results estimated by the equations from the percentage of fat in the several rib cuts used as indicators should not vary greatly except at the limits of the range in the fatness of the animals.

The relations for the prediction of the bone (table 3) in the empty body and carcass from the wholesale and 9–11 rib cuts are linear and have correlation coefficients higher and standard errors of estimate lower than was anticipated on account of individual characteristics. The coefficients for the wholesale rib relation are the higher but on the basis of the Y intercepts and the standard errors of estimate the two cuts are of about equal value for prediction usage.

The relationship between the rib cuts in the percentages of the physical constituents is quite high (table 3 relations 29–41) especially for the fat. In percentage of fat between the whole and edible portions the correlations were +0.998 for the wholesale rib cut and +0.997 for the 9–11 rib cut. Hence the whole and edible portions should serve equally well as fatness indicators, especially in the case of the 9–11 rib cut where the standard error of estimate was but ±0.667.

RELATIONS IN CHEMICAL COMPOSITION

The chemical composition—percentages of moisture, ash, crude protein ($N \times 6.25$) and ether extract—of the whole and of the edible portions of the wholesale rib cut is highly correlated with that of the empty body carcass and edible portion of the carcass (table 4). As the correlation coefficients are all very high and the standard errors of estimate relatively small the relations should be of value for prediction purposes using the composition of the whole or edible portion of the wholesale rib cut as the indicator. The chemical composition of the edible portions of the wholesale and 9–11 rib cuts is highly correlated with that of the corresponding whole portions (table 4).

TABLE 4.—*Relations in chemical constituents*

Relation No.	X	Y	n	r_{xy}	Regression of Y on X	
					Equation	S_{yx} (±)
1	Moisture, wholesale rib	Moisture, empty body	36	0.984	$Y = 0.79970\,X + 14.12032$	1.446
2	Ash, wholesale rib	Ash, empty body	36	.925	$Y = .54723\,X + 1.14747$	.308
3	Crude protein, wholesale rib.	Crude protein, empty body.	36	.948	$Y = .75400\,X + 4.53862$	.731
4	Ether extract, wholesale rib.	Ether extract, empty body.	36	.992	$Y = .79149\,X + 2.20772$	1.311
5	Moisture, edible wholesale rib.	Moisture, empty body	36	.985	$Y = .64010\,X + 19.02780$	1.377
6	Crude protein, edible wholesale rib.	Crude protein, empty body.	36	.943	$Y = .66837\,X + 6.52389$	.762
7	Ether extract, edible wholesale rib.	Ether extract, empty body.	36	.989	$Y = .67616\,X + 4.56741$	1.532
8	Moisture, wholesale rib	Moisture, carcass	36	.991	$Y = .89311\,X + 7.25137$	1.198
9	Ash, wholesale rib	Ash, carcass	36	.953	$Y = .71345\,X + .63730$	.312
10	Crude protein, wholesale rib.	Crude protein, carcass	36	.985	$Y = .84942\,X + 2.24676$	.420
11	Ether extract, wholesale rib.	Ether extract, carcass	36	.994	$Y = .88736\,X + 2.58567$	1.217
12	Moisture, edible wholesale rib.	Moisture, carcass	36	.990	$Y = .71318\,X + 12.83667$	1.253
13	Crude protein, edible wholesale rib.	Crude protein, carcass	36	.981	$Y = .75323\,X + 4.47855$	.480
14	Ether extract, edible wholesale rib.	Ether extract, carcass	36	.992	$Y = .75817\,X + 5.22898$	1.493
15	Moisture, wholesale rib	Moisture, edible carcass.	36	.983	$Y = 1.07820\,X + 2.00290$	2.006
16	Crude protein, wholesale rib.	Crude protein, edible carcass.	36	.985	$Y = .96686\,X - .26467$	.489
17	Ether extract, wholesale rib.	Ether extract, edible carcass.	36	.993	$Y = 1.01546\,X - .33524$	1.641
18	Moisture, edible wholesale rib.	Moisture, edible carcass.	36	.993	$Y = .87065\,X + 8.14647$	1.263
19	Crude protein, edible wholesale rib.	Crude protein, edible carcass.	36	.985	$Y = .86146\,X + 2.20579$	.483
20	Ether extract, edible wholesale rib.	Ether extract, edible carcass.	36	.994	$Y = .87116\,X + 2.61859$	1.456
21	Moisture, edible wholesale rib.	Moisture, wholesale rib.	36	.992	$Y = .79327\,X + 6.58009$	1.218
22	Crude protein, edible wholesale rib.	Crude protein, wholesale rib.	36	.994	$Y = .88567\,X + 2.64602$	.308
23	Ether extract, edible wholesale rib.	Ether extract, wholesale rib.	36	.997	$Y = .85450\,X + 2.97706$	.953
24	Moisture, edible 9–11 rib	Moisture, 9–11 rib	56	.994	$Y = .78144\,X + 7.43330$	.793
25	Crude protein, edible 9–11 rib.	Crude protein, 9–11 rib.	56	.997	$Y = .91536\,X + 2.69842$	.174
26	Ether extract, edible 9–11 rib.	Ether extract, 9–11 rib	56	.998	$Y = .86782\,X + 1.37632$	.523

RELATIONS BETWEEN CHEMICAL AND PHYSICAL CONSTITUENTS

The study of the relations between chemical and physical constituents was limited almost entirely to fatness. It is an observation of practical importance that very high correlation coefficients and relatively low standard errors of estimate were found for the relations between separable fat and ether extract. This is true for all portions examined, namely, empty body, carcass, edible portion of the carcass, wholesale rib, edible wholesale rib, 9–11 rib, and edible 9–11 rib. The relations for the empty body carcass, and edible portion of the carcass are illustrated in figure 1.

The relations between the ether extract in the whole and edible portions of the 9–11 rib cuts and the fat in the empty body, carcass, and edible carcass (table 5 relations 8–13) are very satisfactory, having correlation coefficients ranging from +0.987 to +0.989 and standard errors of estimate from ±1.11 to ±1.41. Figure 2 shows graphically the relations between the 9–11 rib cut and the three gross portions.

TABLE 5.—*Relations between chemical and physical constituents*

Relation No.	X	Y	n	r_{xy}	Regression of Y on X	
					Equation	$S_{yx}(\pm)$
1	Fat, empty body	Ether extract, empty body.	36	0.996	$Y = 1.06089\,X + 3.11131$	0.954
2	Fat, carcass	Ether extract, carcass	36	.994	$Y = 1.01885\,X + 3.76939$	1.291
3	Fat, edible carcass	Ether extract, edible carcass.	36	.994	$Y = 1.06206\,X - .76254$	1.455
4	Fat, wholesale rib	Ether extract, wholesale rib.	36	.988	$Y = 1.00834\,X + 7.40977$	2.076
5	Fat, edible wholesale rib	Ether extract, edible wholesale rib.	36	.990	$Y = 1.03356\,X + 4.08890$	2.140
6	Ether extract, 9–11 rib	Fat, 9–11 rib	56	.992	$Y = .90486\,X - .88734$	1.241
7	Ether extract, edible 9–11 rib.	Fat, edible 9–11 rib	56	.994	$Y = .90454\,X + 1.82599$	1.230
8	Ether extract, 9–11 rib	Fat, empty body	56	.986	$Y = .62433\,X + .88575$	1.121
9	do	Fat, carcass	56	.988	$Y = .74878\,X + 1.36142$	1.249
10	do	Fat, edible carcass	56	.988	$Y = .84457\,X + 2.78782$	1.410
11	Ether extract, edible 9–11 rib.	Fat, empty body	56	.987	$Y = .54260\,X + 1.72666$	1.106
12	do	Fat, carcass	56	.988	$Y = .65064\,X + 2.37270$	1.241
13	do	Fat, edible carcass	56	.989	$Y = .73420\,X + 3.92085$	1.372
14	Fat, edible wholesale rib	Ether extract, wholesale rib.	36	.989	$Y = .88448\,X + 6.45086$	1.928
15	Ether extract, 9–11 rib	Fat, edible 9–11 rib	56	.992	$Y = 1.03899\,X + .46270$	1.434
16	Ether extract, edible 9–11 rib.	Fat, 9–11 rib	56	.993	$Y = .78684\,X + .32139$	1.166
17	Ether extract, eye muscle, 9–11 rib.	Fat, empty body	56	.900	$Y = 4.06166\,X + 3.73293$	3.037
18	do	Fat, carcass	56	.894	$Y = 4.83503\,X + 4.87026$	3.722
19	do	Fat, edible carcass	56	.891	$Y = 5.42977\,X + 6.80732$	4.272
20	Ether extract, fat, 9–11 rib.	Ether extract, 9–11 rib	56	.891	$Y = .50385\,X - 13.69504$	4.994
21	do	Ether extract, edible 9–11 rib.	56	.892	$Y = .58068\,X - 17.37301$	5.720
22	Ether extract, bone, 9–11 rib.	Ether extract, 9–11 rib	56	.676	$Y = 2.07738\,X - 5.16224$	8.113
23	do	Ether extract, edible 9–11 rib.	56	.658	$Y = 2.32610\,X - 6.66734$	9.549
24	Bone, empty body	Ash, empty body	36	.712	$Y = .13204\,X + 2.39445$	.572
25	Bone, carcass	Ash, carcass	36	.678	$Y = .12516\,X + 2.68145$	.759

As should be expected, excellent correlations exist between the percentage of fat in the rib cuts and the ether extract in the corresponding whole or edible portion (table 5, relations 14–16).

Though the correlations found are high, the ether extract of the eye muscle and the separable fat of the 9–11 rib cut are not considered as reliable indicators of fatness (table 5, relations 17–21) as the separable fat and ether extract of the rib cuts. The standard errors of
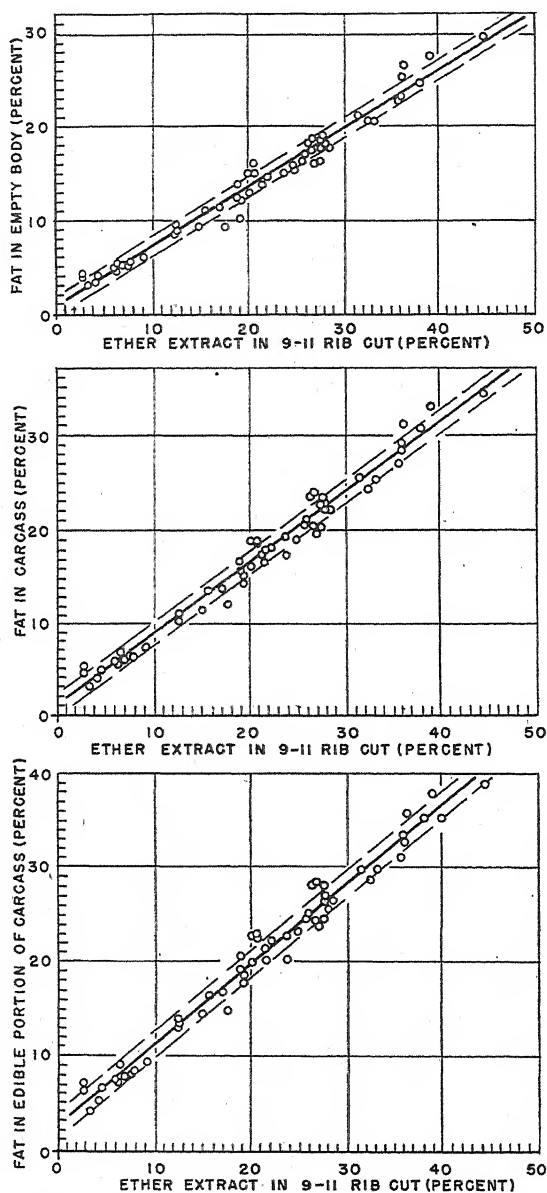
FIGURE 1.—Relations between the percentages of fat and ether extract in the empty body, carcass, and edible portion of carcass (36 Missouri steers and cows). Empty body: $Y = 1.06089\ X + 3.11131$; $r_{xy} = +0.996$; $S_{yx} = \pm 0.954$.   Carcass: $Y = 1.01885\ X + 3.76939$; $r_{xy} = +0.994$; $S_{xy} = \pm 1.291$.   Edible carcass: $Y = 1.06206\ X - 0.76254$; $r_{xy} = +0.994$; $S_{yx} = \pm 1.455$.
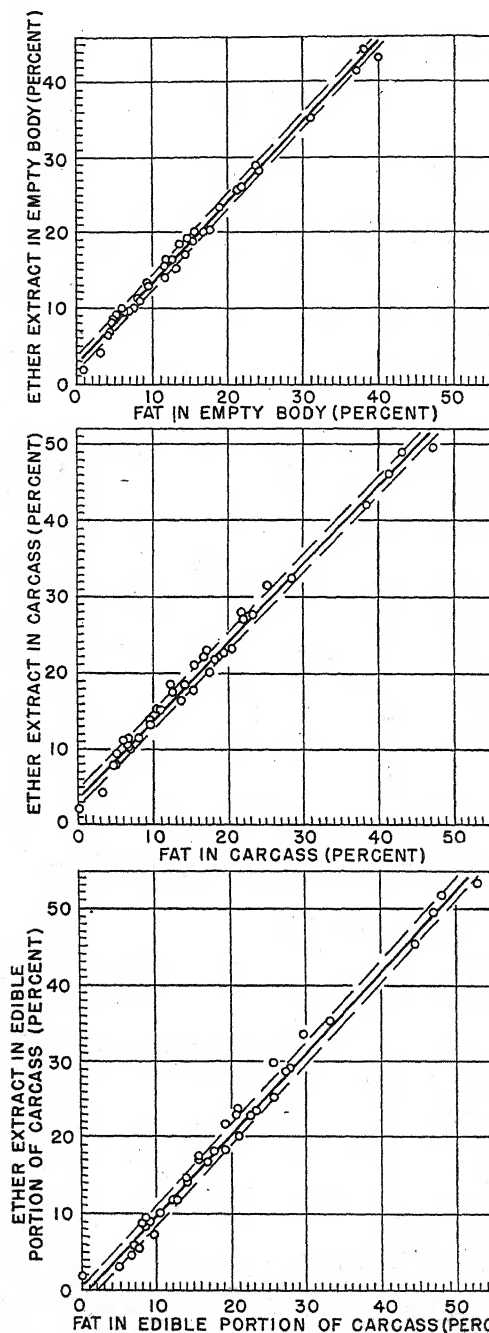
FIGURE 2.—Relations between the percentages of ether extract in the 9–11 rib cut and fat in empty body, carcass, and edible carcass (56 North Dakota steers). Empty body: $Y=0.62433\ X+0.88575$; $r_{xy}=+0.986$; $S_{yx}=\pm1.121$. Carcass: $Y=0.74878\ \ X+1.36142$; $r_{xy}=+0.988$; $S_{yx}=\pm1.249$. Edible carcass: $Y=0.84457\ X+2.78782$; $r_{xy}=+0.988$; $S_{yx}=\pm1.410$.

estimate are high.   A narrow range in ether extract (6.35) in the eye muscle corresponded to a relatively wide range in fat in the empty body (26.37), carcass (31.00), and edible portion of the carcass (34.73).

The ether extract in the bone of the 9–11 rib cut is not a reliable indicator of fatness (table 5, relations 22–23), as the standard error of estimate is too high for satisfactory prediction.

The percentages of bone and ash in the empty body and carcass are not satisfactory indicators of each other.   Though the correlation coefficients are high the standard errors of estimate are relatively large proportions of the respective substances present (table 5, relations 24–25) for the relations to be adequate for prediction purposes.

The dressing percentage has long been used as a general indicator of fatness.   It has special significance to the packer in his operations. However, it is a function of other factors than fatness.   Individual and breed differences in percentages of offal and its constituents,
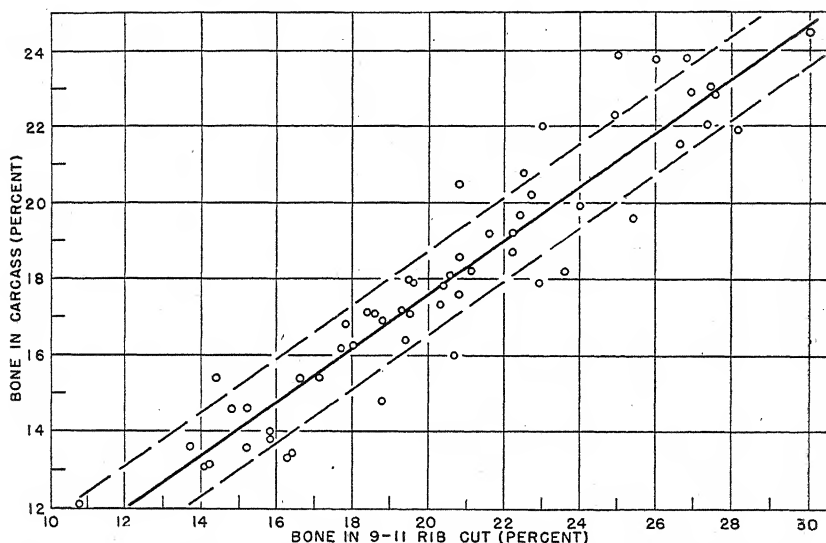
FIGURE 3.—Relation between percentage of fat in the empty body and the empty-body dressing percentage (92 animals): $Y = 0.25541X + 61.44850$; $r_{xy} = +0.716$; $S_{yx} = \pm 2.055$.

especially of the head, feet, and hide, are of material significance.   Of even greater significance are variations in weights of the digestive fill. Though the relations between the percentages of fat and of ether extract and the empty body dress have relatively high correlation coefficients, the standard errors of estimate are rather high for accurate prediction (table 6).   The empty-body weight has been used to eliminate variations in digestive fill.   The empty-body dressing percentage used is the percentage of warm carcass of the empty body.   Only the simple correlations were calculated.   It is noted that wide ranges in fatness occur for the same dressing percentages (fig. 3), and it must follow that when used, the dressing percentage may only be considered a general indicator for wide ranges in fatness.

TABLE 6.—*Relations between fatness and empty-body dressing percentage*

Relation No.	X	Y	n	r_{xy}	Equation	S_{yx} (±)
	Regression of Y on X:					
1a____	Fat in empty body_____	Empty-body dress	92	0.716	$Y = 0.25541\ X + 61.44850$	2.055
2a____	Fat in carcass_____	____do_____	92	.714	$Y = .21592\ X + 61.41082$	2.062
3a____	Fat in edible carcass_____	____do_____	92	.707	$Y = .19231\ X + 61.15838$	2.081
4a____	Ether extract in empty body.	____do_____	36	.705	$Y = .24734\ X + 60.77816$	2.695
5a____	Ether extract in carcass____	____do_____	36	.709	$Y = .22238\ X + 60.71873$	2.680
6a____	Ether extract in edible carcass.	____do_____	36	.699	$Y = .19114\ X + 60.42296$	2.718
						S_{xy} (±)
	Regression of X on Y:					
1b____	Fat in empty body_____	____do_____	92	.716	$X = 2.00981\ Y - 116.57247$	5.765
2b____	Fat in carcass_____	____do_____	92	.714	$X = 2.36219\ Y - 136.72977$	6.821
3b____	Fat in edible carcass_____	____do_____	92	.707	$X = 2.60502\ Y - 149.13212$	7.659
4b____	Ether extract in empty body.	____do_____	36	.705	$X = 2.01179\ Y - 113.16029$	7.685
5b____	Ether extract in carcass____	____do_____	36	.709	$X = 2.26304\ Y - 127.25047$	8.548
6b____	Ether extract in edible carcass.	____do_____	36	.699	$X = 2.55730\ Y - 146.79776$	9.942

CHEMICAL COMPOSITION ON ETHER EXTRACT-FREE BASIS

It has been suggested that the chemical composition on the ether free basis may be used as a practical constant (*4, 9*), assuming that the change after reaching chemical maturity due to age is relatively slight. However, it has also been indicated that this composition is a function of fatness in mature animals (*1*).

If the variations in the chemical composition on the ether extract-free basis due to age and fatness should be smaller than the inherent errors of experimental methods, the averages of the results available for study should be satisfactory as practical constants. If such is the case, then it remains in estimating the chemical composition but to determine the percentage of ether extract.

The chemical composition on the ether extract-free basis of the empty body and the whole and edible portions of the carcass and whole-sale rib cut of the Missouri animals, and the whole and edible portion of the 9–11 rib cut of the North Dakota steers is summarized in table 7. The statistical analysis of the data is given in the same table. Considering the wide variations in age, weight, fatness, and breeding of the Missouri animals, the variations observed are relatively small, especially in the edible portions.

The relation between the ether extract-free composition and age in months is shown graphically in figure 4 for each of the three major portions of the animal. The lines drawn represent the means and the regression equations. That there is a slight decrease in moisture and increase in ash and protein with age is indicated by the graphs. The ash, however, in the edible portion of the carcass varies so slightly that the mean should be better than a practical constant. The data for the three aged Jersey cows appear to conform in general to the change-with-age tendency in the carcass and edible portion of the carcass but not in the case of the empty body.
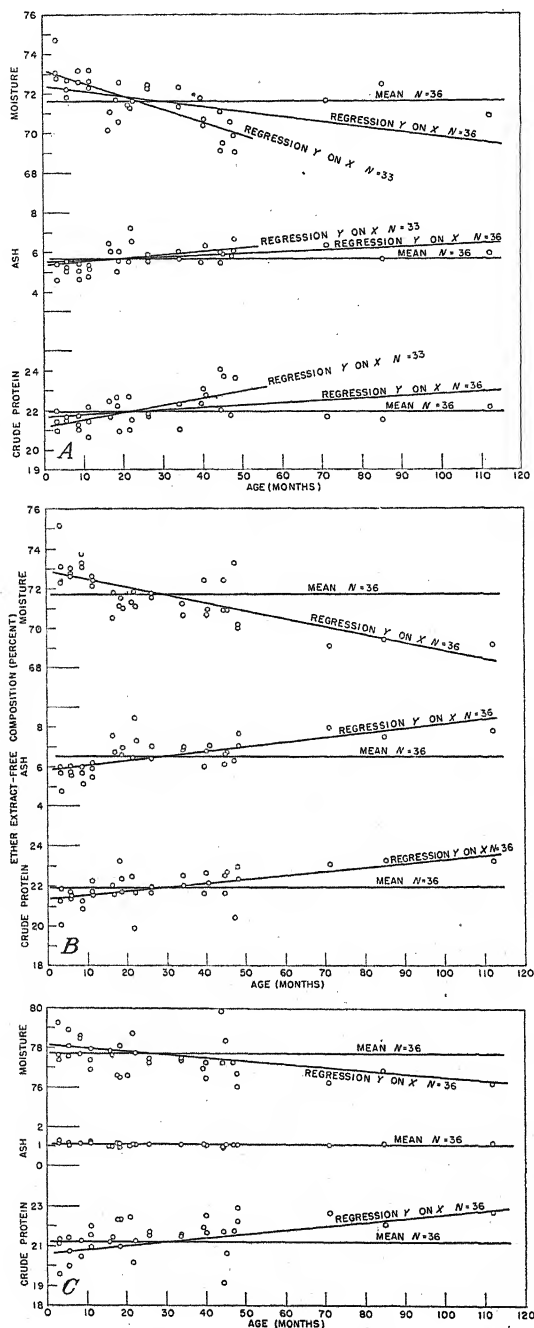
FIGURE 4.—Relation between chemical composition on the ether extract-free basis of the empty body (*A*), carcass (*B*), and edible portion of the carcass (*C*), and age in months. The lines represent the means and the regression equations tables 7 and 8).

TABLE 7.—*Range in and mean of ether extract-free chemical composition and correlation of this composition with age in months of animals and fatness (percentage of ether extract)*

	Range	Mean	Standard error of mean (±)	Standard deviation (±)	Simple correlation				Partial correlation	
					With age (months)		With percent ether extract		With age (months)	With percent ether extract
					Correlation coefficient	Regression coefficient [1]	Correlation coefficient	Regression coefficient [2]	Correlation coefficient	Correlation coefficient
Missouri steers and cows (36 animals):										
Empty body:		*Percent*								
Moisture	69.02–74.74	71.662	0.205	1.245	−0.480	−0.02456	−0.280	−0.03266	−0.408	−0.048
Ash	4.49–7.15	5.637	.096	.580	+.431	+.01027	−.046	−.00254	+.528	−.341
Crude protein	20.57–24.00	21.951	.138	.838	+.367	+.01264	+.225	+.01767	+.301	+.048
Moisture: protein ratio	2.88–3.57	3.270	.028	.170	−.424	−.00296	−.264	−.00420	−.349	−.062
Carcass:										
Moisture	69.06–75.13	71.665	.220	1.337	−.740	−.04072	−.174	−.01953	−.763	−.321
Ash	4.83–8.44	6.491	.134	.813	+.617	+.02064	+.025	+.0170	+.694	−.403
Crude protein	19.78–23.23	21.843	.136	.829	+.589	+.02008	+.257	+.01783	+.549	−.044
Moisture: protein ratio	2.97–3.75	3.287	.030	.180	−.576	−.00428	−.239	−.00362	−.542	−.059
Edible portion of carcass:										
Moisture	76.20–79.69	77.721	.130	.795	−.572	−.01870	−.122	−.00709	−.593	−.224
Ash	.94–1.23	1.069	.010	.061	−.142	−.00036				•
Crude protein	19.28–22.70	21.210	.129	.789	+.588	+.01906	+.146	+.00844	+.599	+.203
Moisture: protein ratio	3.36–4.13	3.669	.028	.174	−.559	−.00402	−.131	−.00108	−.573	+.199
Wholesale rib cut:										
Moisture	66.29–74.53	69.714	.291	1.769						
Ash	5.30–10.28	7.987	.209	1.274						
Crude protein	20.17–23.65	22.299	.136	.827						
Edible portion of wholesale rib:										
Moisture	76.03–79.93	77.504	.145	.884						
Ash	.91–1.24	1.009	.012	.071						
Crude protein	19.16–22.91	21.427	.145	.880						
North Dakota steers (56 animals):										
9–11 rib cut:										
Moisture	65.21–70.20	67.908	.210	1.263						
Ash	6.58–10.50	8.543	.164	.992						
Crude protein	21.62–24.60	23.549	.098	.593						
Edible portion of 9–11 rib cut:										
Moisture	75.23–78.26	76.435	.108	.649						
Ash	1.03–1.16	1.098	.005	.029						
Crude protein	20.62–23.61	22.467	.106	.637						

[1] Regression of composition on age in months.
[2] Regression of composition on percent ether extract.

Simple correlations of the ether extract-free composition with age in months have negative coefficients for the percentage of moisture (table 7).   The ash coefficients are positive for the empty body and carcass but they are negative for the edible portion of the carcass. The crude protein coefficients are positive.   The regression coefficients of the composition on age are too small for the relations to be highly satisfactory for prediction purposes.

The simple correlations of the ether extract-free composition with the percent of ether extract in the original portions have regression coefficients in most cases which are insignificantly small (table 7).

Partial correlation coefficients (table 7) calculated for the relation between the ether extract-free composition and age and percentage ether extract in the original portions show that the tendency for change in composition is largely attributable to age and not to fatness. The partial coefficients with age are negative for moisture and positive for ash and protein.

Some have attached importance to the moisture-protein ratio. With a tendency for percentage of moisture to decrease and percentage of protein to increase with age there should be a decrease in the ratio with age.   The standard errors of the means and the standard deviations (table 7) are relatively small.   The ratios are negatively correlated with age.   The regression coefficients of the ratios on age are relatively quite small.   Partial correlation of the ratios gives negative coefficients with age.

Murray (*9*) concluded that the ratio of protein to ash does not alter with the age of the animal but that it may be influenced to a certain extent by the food, and he gave the mean value of the ratio for his data as 4.392 ± 0.215 for the empty body.   For the present data (36 animals) the ratio ranged from 2.94 to 4.70 and averaged 3.93 with a standard deviation of ± 0.371 for the empty body.   For the carcass it ranged from 2.91 to 4.18 and averaged 3.41 with a standard deviation of ± 0.388 and for the edible portion of the carcass it ranged from 16.82 to 22.31 and averaged 19.90 with a standard deviation of $+1.26$.

It was noted that the composition on the ether extract-free basis of the empty body for the 3 aged Jersey cows did not conform to the continuation of the change in composition of the 33 steers (fig. 4). For this reason the statistical data for the 33 steers were calculated for comparison with those of the entire group including the 3 cows (table 8).   There are but small differences in means and standard deviations between the 2 groups.   For the empty body, the correlation coefficients are higher, the regression coefficients higher, and the standard errors of estimate lower for the group of 33 steers.   For the carcass and edible portion of the carcass the correlation coefficients are higher for the entire group of 36 animals, though there were rather small differences in the regression coefficients and the standard errors of estimate.   It is noted that there is but small reduction from the standard deviation to the standard error of estimate.

Composition on the ether extract-free basis may be considered a practical constant for some purposes.   However, to a certain extent it is a function of age.   Using the regression of the composition on age, the composition at 10, 20, 30, 40, and 50 months of age was calculated (table 9) for the 33 steers and also for the 36 steers and cows.

The data and the graphs (fig. 4) indicate that the results calculated according to the regression should have practical use in estimating the chemical composition when the fatness determined as ether extract is known.

TABLE 8.—*Comparison of statistical analysis of composition on the ether extract-free basis of the 33 steers with the 33 steers and 3 cows (Missouri animals) and the correlation of the composition with age in months*

Y	N=33 steers, Missouri					
	Mean	Standard error of mean ($\pm$)	Standard deviation ($\pm$)	r_{xy}	b_{yx}	S_{yx} ($\pm$)
Empty body:	*Percent*					
Moisture	71.663	0.179	1.045	−0.764	−0.06337	0.840
Ash	5.609	.102	.591	+.533	+.02037	.508
Crude protein	21.970	.150	.870	+.677	+.03804	.649
Carcass:						
Moisture	71.891	.180	1.047	−.606	−.04494	.928
Ash	6.384	.130	.759	+.516	+.02534	.660
Crude protein	21.724	.130	.758	+.400	+.01961	.706
Edible portion of carcass:						
Moisture	77.851	.119	.692	−.284	−.01273	.674
Ash	1.066	.011	.063	−.484	−.00196	.056
Crude protein	21.083	.118	.692	+.329	+.01469	.663

Y	N=33 steers and 3 cows=36 animals, Missouri					
	Mean	Standard error of mean ($\pm$)	Standard deviation ($\pm$)	r_{xy}	b_{yx}	S_{yx} ($\pm$)
Empty body:	*Percent*					
Moisture	71.662	0.025	1.245	−0.480	−0.02456	1.107
Ash	5.637	.096	.580	+.431	+.01027	.530
Crude protein	21.951	.138	.830	+.367	+.01264	.790
Carcass:						
Moisture	71.665	.220	1.337	−.740	−.04072	.910
Ash	6.491	.134	.813	+.617	+.02064	.649
Crude protein	21.843	.136	.829	+.589	+.02008	.679
Edible portion of carcass:						
Moisture	77.721	.130	.795	−.572	−.01870	.661
Ash	1.069	.010	.061	−.142	−.00036	.061
Crude protein	21.210	.129	.789	+.588	+.01906	.647

X=Age in months (N=33, mean=22.818, standard deviation=15.282; N=36, mean=28.361, standard deviation=24.003).
r_{xy}=Correlation coefficient.
b_{yx}=Regression coefficient of composition on age in months.
S_{yx}=Standard error of estimate of composition.

TABLE 9.—*Mean composition and composition by regression on age in months for the empty body, carcass, and edible portion of carcass on ether extract-free basis*

Item	For 33 Steers, Missouri							
	Mean [1]	Standard ($\pm$) deviation	Composition by regression on age (months)					Standard error of estimate ($\pm$)
			10	20	30	40	50	
Empty body:	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Moisture	71.663	1.045	72.475	71.841	71.208	70.574	69.941	0.840
Ash	5.609	.591	5.348	5.552	5.755	5.959	6.163	.508
Crude protein	21.970	.870	21.483	21.863	22.243	22.624	23.004	.649
Carcass:								
Moisture	71.891	1.047	72.467	72.017	71.568	71.119	70.669	.928
Ash	6.384	.759	6.059	6.313	6.566	6.819	7.073	.660
Crude protein	21.724	.758	21.473	21.669	21.865	22.061	22.257	.706
Edible portion of carcass:								
Moisture	77.851	.692	78.014	77.887	77.760	77.632	77.505	.674
Ash	1.066	.063	1.091	1.072	1.052	1.032	1.013	.056
Crude protein	21.083	.692	20.895	21.042	21.189	21.335	21.482	.663

[1] Mean composition at mean age of 22.818 months.

TABLE 9.—*Mean composition and composition by regression on age in months for the empty body, carcass, and edible portion of carcass on other extract-free basis*—Continued.

Item	For 33 steers and 3 cows (36 animals), Missouri							
	Mean [2]	Standard deviation (±)	Composition by regression on age (months)					Standard error of estimate (±)
			10	20	30	40	50	
Empty body:	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Moisture	71.662	1.245	72.113	71.867	71.622	71.376	71.131	1.107
Ash	5.637	.580	5.449	5.551	5.654	5.757	5.859	.530
Crude protein	21.951	.838	21.719	21.845	21.972	22.098	22.225	.790
Carcass:								
Moisture	71.665	1.337	72.413	72.005	71.598	71.191	70.784	.910
Ash	6.491	.813	6.112	6.319	6.525	6.731	6.938	.649
Crude protein	21.843	.829	21.475	21.675	21.876	22.077	22.277	.679
Edible portion of carcass:								
Moisture	77.721	.795	78.064	77.877	77.690	77.503	77.316	.661
Ash	1.069	.061	1.075	1.072	1.068	1.065	1.061	.061
Crude protein	21.210	.789	20.861	21.051	21.241	21.432	21.622	.647

[2] Mean composition at mean age of 28.361 months.

The slightly lower standard errors of estimate (table 8) indicate the use of the average composition on the ether extract-free basis by regression for the 33 steers as practical constants for the ages indicated for the empty body, and that for the 36 steers and cows for the carcass and edible portion of the carcass (table 9). The use of these values should be preferred to the use of the mean values of the ether extract-free composition as practical constants as they take into account the small influence of age.

The lack of conformity of the three aged Jersey cows in the case of the empty body apparently is due to the maintenance of a high moisture content in the offal. There is a possibility that this may be related to sex, pregnancy, and lactation.

ESTIMATION OF ASH FROM ETHER EXTRACT IN EDIBLE PORTIONS

The observation that age does not significantly influence the percentage of ash in the ether extract-free edible portion of the carcass, indicated by the low standard deviation, low regression coefficient, and the similarity between the standard deviation and standard error of estimate (table 8), suggests that in the analysis of the edible portions the percentage of ash may be estimated as accurately from the percentage of ether extract as the ash may be determined by laboratory means. The summary analysis of the percentage of ash in five portions not containing bone and the relations between the percentages of ash and ether extract in these portions derived from the mean percentages of ash on the ether extract-free basis, assuming a uniform regression for infiltration of ether extract from 0 to 100 percent, show a similarity in those portions containing lean (table 10). The summary relations for the three edible portions and for the five portions containing lean were identical. However, there is a larger mean percentage of ash in the ether extract-free fat or separable adipose tissue of the 9–11 rib cut than in the edible portions containing lean. The difference is larger than may possibly be accounted for by error in analysis and accidental inclusion of some bone in separating the fat.

TABLE 10.—*Relation between percentage of ash and percentage of ether extract in some edible portions derived from the mean percentage of ash on the ether extract-free basis*

| Relation No. | Animal portion | n | Ash (ether extract-free basis) | | | | | Equation [1] |
			Lowest	Highest	Range	Standard deviation (±)	Mean	
			Percent	*Percent*			*Percent*	
1	Edible carcass	36	0.94	1.23	0.29	0.061	1.069	$Y=1.069-0.01069\ X$
2	Edible wholesale rib	36	.91	1.24	.33	.071	1.069	$Y=1.069-.01069\ X$
3	Edible 9–11 rib	56	1.03	1.16	.13	.029	1.098	$Y=1.098-.01098\ X$
4	Eye muscle, 9–11 rib	56	1.04	1.20	.16	.029	1.087	$Y=1.087-.01087\ X$
5	Other lean, 9–11 rib	56	.96	1.16	.20	.036	1.080	$Y=1.080-.01080\ X$
6	Fat, 9–11 rib	56	1.05	1.73	.68	.163	1.315	$Y=1.315-.01315\ X$
7	Edible portions (1, 2, 3).	128	.91	1.24	.33	.054	1.082	$Y=1.082-.01082\ X$
8	Portions with lean (1, 2, 3, 4, 5).	240	.91	1.24	.33	.045	1.082	$Y=1.082-.01082\ X$

[1] X= Percent ether extract; Y=percent ash.

The relations derived by correlation analysis (table 11) are similar to those derived from the mean percentages of ash (table 10), and practical results calculated from them vary but little except in the case of the fat of the 9–11 rib cut. The standard errors of estimate are approximately the variation usually accepted on the duplication of analysis. If used practically, it is suggested that relation 8, table 11, be used for edible portions containing lean, and relation 6, table 11, be used for fat or separable adipose tissue. The data used do not indicate possibility of variation in the percentage of ash in the edible portion of the various cuts and in the adipose tissue of the various parts of the carcass.

ESTIMATION OF FATNESS

The fundamental measure of fatness is the percentage of ether extract or true chemical fat. It is evident from the data presented that there is a possibility of predicting or estimating the percentage of ether extract in the empty body of cattle by using as an indicator the percentage of ether extract or separable fat in the whole and edible portions of the wholesale or 9–11 rib cuts.

The relations for estimation from the percentage of ether extract and fat of the whole and edible portion of the wholesale rib cut were calculated directly from the compositional data for the 36 Missouri animals (relations 1, 2, 5, 7, 11, 12, 15, 17, 21, 22, 25, and 27, table 12). The correlation coefficients and the standard errors of estimate were calculated for these relations.

The Y intercepts for the equations when using the separable fat of the whole and edible portion of the wholesale rib cut are relatively large (relations 5, 7, 15, 17, 25, and 27, table 12). For this reason similar relations were derived indirectly by combination of the relations between the fat in the wholesale rib cuts and the fat in the empty body, carcass, and edible carcass with the relations between the fat and ether extract in the empty body, carcass, and edible portion of the carcass (relations 6, 8, 16, 18, 26, and 28, table 12). The relations combined in the derivations are indicated in the footnote to table 12.

TABLE 11.—*Relation between the percentage of ash and the percentage of ether extract in some edible portions determined by correlation analysis*

Rela- tion No.	Animal portion	n	X (ether extract)					Y (ash)					Correlation coefficient r_{xy}	Regression of Y on X[1]	S_{yz} (±)
			Lowest	Highest	Range	Standard deviation (±)	Mean	Lowest	Highest	Range	Standard deviation (±)	Mean			
			Percent	*Percent*			*Percent*	*Percent*	*Percent*			*Percent*			
1	Edible carcass	36	1.89	53.32	51.43	13.71	20.11	0.48	1.16	0.68	0.43	0.86	−0.953	Y=1.0910−0.01164 X	0.050
2	Edible wholesale rib	36	2.09	59.82	57.73	15.66	20.08	.41	1.16	.75	.19	.86	−.957	Y=1.0921−.01166 X	.055
3	Edible 9–11 rib	56	1.84	48.58	46.74	12.57	23.13	.57	1.09	.52	.13	.84	−.985	Y=1.0844−.01048 X	.022
4	Eye muscle, 9–11 rib	56	.39	6.74	6.35	1.53	2.60	1.00	1.16	.16	.03	1.06	−.485	Y=1.0838−.00995 X	.027
5	Other lean, 9–11 rib	56	1.77	21.28	19.51	4.75	10.53	.83	1.10	.27	.07	.97	−.904	Y=1.0966−.01219 X	.027
6	Fat, 9–11 rib	56	16.18	88.86	72.68	19.32	69.75	.15	1.02	.87	.22	.38	−.977	Y=1.1720−.01131 X	.047
7	Edible portions (1, 2, 3)	128	1.84	59.82	57.98	13.12	21.42	.41	1.16	.75	.16	.85	−.961	Y=1.0897−.01115 X	.044
8	Portions with lean (1, 2, 3, 4, 5)	240	.39	59.82	59.43	14.02	14.49	.41	1.16	.75	.15	.94	−.968	Y=1.0960−.01112 X	.037

[1] X= Percent ether extract; y=percent ash.

The relations so derived had lower Y intercepts and very similar regression coefficients as compared to the relations calculated from the experimental data.

TABLE 12.—*Relations of the ether extract in the empty body, carcass, and edible portion of the carcass to the chemical and physical fat in the whole and edible portion of the wholesale and 9-11 rib cuts*

Relation No.	X	Y	n[1]	rxy	Regression of Y on X	
					Equation	Yx ±
1	Ether extract, wholesale rib.	Ether extract, empty body.	36	0.992	$Y = 0.79149 X + 2.20772$	1.311
2	Ether extract, edible wholesale rib.	do	36	.989	$Y = .67617 X + 4.56741$	1.532
3	Ether extract, 9-11 rib	do	(1)	.983	$Y = .66234 X + 4.05099$	
4	Ether extract, edible 9-11 rib.	do	(2)	.983	$Y = .57564 X + 4.94311$	
5	Fat, wholesale rib	do	36	.976	$Y = .79457 X + 8.11691$	2.327
6	do	do	(3)	.969	$Y = .77865 X + 6.63270$	
7	Fat, edible wholesale rib	do	36	.992	$Y = .69751 X + 7.35292$	2.207
8	do	do	(4)	.966	$Y = .67466 X + 5.87784$	
9	Fat, 9-11 rib	do	(5)	.981	$Y = .72534 X + 4.82360$	
10	Fat, edible 9-11 rib	do	(6)	.977	$Y = .62885 X + 3.95249$	
11	Ether extract, wholesale rib.	Ether extract, carcass	36	.994	$Y = .88736 X + 2.58567$	1.217
12	Ether extract, edible wholesale rib.	do	36	.992	$Y = .75817 X + 5.22898$	1.493
13	Ether extract, 9-11 rib.	do	(7)	.983	$Y = .76289 X + 5.15647$	
14	Ether extract, edible 9-11 rib.	do	(8)	.983	$Y = .66290 X + 6.18682$	
15	Fat, wholesale rib	do	36	.980	$Y = .89249 X + 9.18945$	2.370
16	do	do	(9)	.976	$Y = .88942 X + 7.87455$	
17	Fat, edible wholesale rib	do	36	.983	$Y = .78381 X + 8.32603$	2.187
18	do	do	(10)	.974	$Y = .77212 X + 6.98505$	
19	Fat, 9-11 rib	do	(11)	.979	$Y = .83315 X + 6.08894$	
20	Fat, edible 9-11 rib	do	(12)	.976	$Y = .72365 X + 5.05824$	
21	Ether extract, wholesale rib.	Ether extract, edible carcass.	36	.993	$Y = 1.01546 X - .33524$	1.641
22	Ether extract, edible wholesale rib.	do	36	.994	$Y = .87116 X + 2.61859$	1.456
23	Ether extract, 9-11 rib.	do	(13)	.983	$Y = .89698 X + 2.19829$	
24	Ether extract, edible 9-11 rib.	do	(14)	.983	$Y = .77976 X + 3.40164$	
25	Fat, wholesale rib	do	36	.980	$Y = 1.02339 X + 7.19589$	2.708
26	do	do	(15)	.973	$Y = 1.02829 X + 5.62564$	
27	Fat, edible wholesale rib	do	36	.984	$Y = .89974 X + 6.19082$	2.422
28	do	do	(16)	.974	$Y = .89476 X + 4.55921$	
29	Fat, 9-11 rib	do	(17)	.977	$Y = .97803 X + 3.32361$	
30	Fat, edible 9-11 rib	do	(18)	.977	$Y = .85136 X + 2.07101$	

[1] Parentheses indicate derived relations, see text.

Relations derived by combination of 2 other relations. Correlation coefficients calculated as square root of the product of the regression coefficients of derived relations.

Relations combined: (1) 8 and 1, table 5; (2) 11 and 1, table 5; (3) 5, table 3, and 1, table 5; (4) 6, table 3, and 1, table 5; (5) 7, table 3, and 1, table 5; (6) 8, table 3, and 1, table 5; (7) 9 and 2, table 5; (8) 12 and 2, table 5; (9) 15, table 3, and 2, table 5; (10) 16, table 3, and 2, table 5; (11) 18, table 3 and 2, table 5; (12) 10 and 3, table 5; (14) 13 and 3, table 5; (15) 25, table 3, and 2, table 5; (16) 26, table 3, and 3, table 5; (17) 27, table 3, and 3, table 5; (18) 28, table 3, and 3, table 5.

It was not possible to calculate directly the relations between the fat and ether extract of the whole and edible portion of the 9–11 rib cut and the ether extract of the empty body, carcass, and edible portion of the carcass as no analyses were made to give the chemical composition of the 56 North Dakota steers. However, relations were derived indirectly, by using the relations calculated between the percentage of ether extract and the percentage of fat in the empty body, carcass, and edible carcass from the experimental data for the 36 Missouri animals and the relations calculated between the ether extract in the whole and edible portion of the 9–11 rib cut and the fat

in the empty body, carcass, and edible carcass from the experimental data for the 56 North Dakota steers (relations 3, 4, 9, 10, 13, 14, 19, 20, 23, 24, 29, and 30, table 12). The correlation coefficients were calculated as the square root of the product of the regression coefficients of the two regression equations derived in each case.

The procedure used in the indirect derivation of the relations from the two sets of data does not yield "least-square" values for these statistics. It can be shown, however, that under certain conditions this procedure will yield regression constants and correlation coefficients closely approximating those which would have been obtained by the method of least squares had the data been complete. These conditions require that the regression coefficients of the two sets of data be representative of the population and that the correlation coefficients for both sets of data be high. As the animals used may be considered representative of the population and the correlation coefficients for the two sets of data used in each case in obtaining the derived relations were very high, the derived relations and coefficients are considered very close approximations. In subsequent discussion the derived statistics will be treated as least-square values. No method was found for estimating the standard errors of estimate for the derived relations.

The standard errors of estimate and prediction value of the derived relations should be relatively satisfactory since those of the relations from which they were derived were reasonably satisfactory. Tests on additional data showed closer agreement with experimentally determined percentages of ether extract in the three gross portions for values estimated by use of the derived 9–11 rib cut relations than for values estimated by use of the directly calculated wholesale rib cut relations.

The correlation coefficients are all extremely high. For the estimation of the ether extract in the gross portions, the correlations coefficients are in general slightly higher for the ether extract than for the fat relations of the several rib cuts. In the case of the ether extract in the wholesale rib cuts, on the basis of standard errors of estimate, the whole portion appears to be the better indicator of the percentage of ether extract in the empty body and carcass, but for the edible portion of the carcass the edible portion of the wholesale rib cut is the better.

There are considerable variations in the Y intercepts, indicating considerable variation in the percentage of ether extract in the gross portions for zero values in the ether extract and fat groups of indicators. Zero fat content must not be confused as indicating zero ether extract content. Some ether extract is always present when no separable fat is present in the rib cut. This is particularly true in the whole portions containing bone on account of the marrow.

WORK METHODS AND APPLICATION OF SELECTED RELATIONS

In animal feeding trials where more detailed estimates of performance are desirable or needed than the change in live weight, a combination of growth and fattening, and the relative fatness as roughly indicated by the dressing percentages, it is suggested that the fatness and composition of the empty body and carcass be esti-

mated from the composition of the 9–11 rib cut. For this procedure it is necessary to obtain the weights of the empty body and warm carcass and the wholesale rib cut for analysis.

The empty body weight may easily be obtained by deducting the weight of the digestive contents from the slaughter live weight. The weight of the digestive contents may be obtained as the difference between the gross and net weight of the digestive organs, stomachs, intestines, and urinary bladder as they are removed from the animal on the slaughter floor. The warm carcass weight is obtained before the carcass is washed and shrouded.

In order to relate the data back to the pasture or feed-lot live weight the loss from this weight to the slaughter live weight must be noted. This loss and the digestive contents give the total reduction from the pasture or feed-lot live weight to the empty-body weight.

For the physical and chemical analysis of the indicator cut, the wholesale rib cut (seven ribs), cut according to the standard methods of cutting, is obtained. This method is based on the anatomy of the animal. The side is cut into quarters, leaving one rib (the thirteenth) on the hind quarter. The cut made in separating the wholesale rib cut from the plate is started at the point of intersection on the twelfth rib of a perpendicular erected on the straight line from the tip of the chine bone to the cartilage of the thirteenth rib, where cut in separating the quarters, at a point 61.5 percent of this distance from the tip of the chine bone. The 9–11 rib cut is then taken from the wholesale rib cut crowding the forward ribs with the knife in making the two cuttings. Accurate and uniform care must be taken in the separation of this rib cut into lean, fat, and bone. Accurate weights of the separated portions must be taken. The lean, fat, and bone samples should be analyzed for moisture, ash, protein, and ether extract, of which determinations the ether extract is the most important constituent for consideration. The chemical composition of the whole and edible portion is calculated from the weights and the composition of the portions.

The proposed methods are based on the warm empty body, the warm carcass, and the edible portion of the warm carcass. The cooler shrinkage of the rib cut should be considered as moisture and as proportional to that of the whole carcass. The chemical composition of the 9–11 rib cut and its parts, taken for chemical analysis, should be corrected for this moisture loss, distributing the loss proportionally between the lean, fat, and bone.

For predicting the percentage physical composition of the edible portion of the carcass only the relation between the fat in the edible portion of the 9–11 rib (X) and the fat in the edible portion of the carcass (Y) (relation 28, table 3) (fig. 5) is needed: $Y = 0.80161 X + 2.66798$; $r_{xy} = +0.982$; $S_{yx} = \pm 1.748$. The percentage of lean in the edible portion of the carcass may be calculated by using relation 24, table 3, but is more simply obtained as being 100 percent minus the estimated percentage of fat. The results will be the same in either case.

In predicting the percentage physical composition of the carcass only the percentage of bone is needed in addition to the percentage of lean and fat in the edible portion. The percentage of bone in the carcass (Y) is estimated from the percentage of bone in the 9–11 rib cut (X), by using relation 20, table 3 (fig. 6), $Y = 0.70750 X + 3.47863$;

$r_{xy} = +0.941$; $S_{yx} = \pm 1.120$.   The percentages of the lean and fat in the edible portion of the carcass are then calculated to the percentage of lean and fat in the whole carcass including the bone.
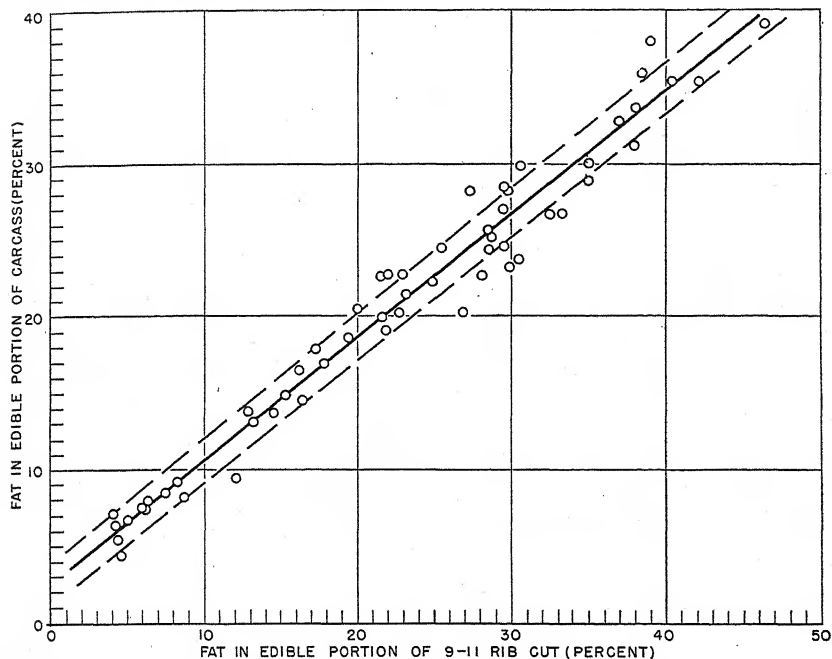
FIGURE 5.—Relation between the percentage of fat in the edible portion of the 9–11 rib cut and the percentage of fat in the edible portion of the carcass. $Y = 0.80161\ X + 2.66798$; $r_{xy} = +0.982$; $S_{yx} = \pm 1.748$.
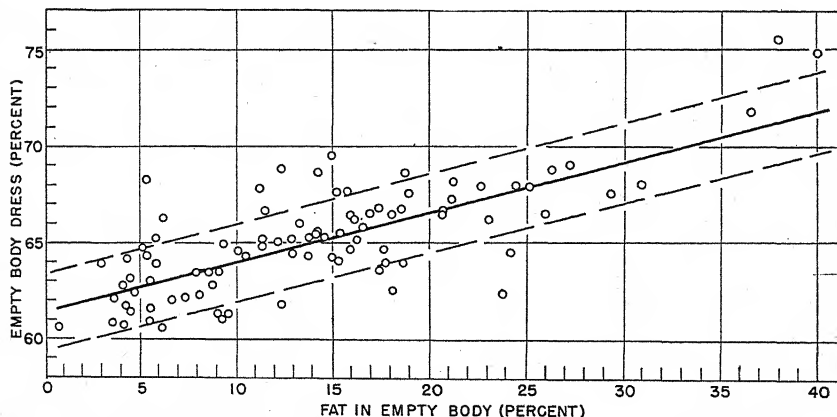
FIGURE 6.—Relation between the percentage of bone in the 9–11 rib cut and the percentage of bone in the carcass. $Y = 0.70750\ X + 3.47863$; $r_{xy} = +0.941$; $S_{yx} = \pm 1.120$.

The correlation coefficient of the lean in the 9–11 rib cut with that in the empty body is not high as compared to other coefficients observed in this study.   Perhaps this is because the weight of the lean

in the empty body is not materially increased over that in the carcass. For a series of 58 steers the weights of the lean in the empty body ranged from 1.06 to 2.26 and averaged 1.644 percent more than the weight of the lean in the carcass. The standard deviation of the data was ±0.248. This suggests that where the weight or percentage of the lean in the empty body is needed the weight should be taken as 1.0164 times the weight of the lean in the carcass. This procedure should not introduce serious error and does not yield data inconsistent with the estimated results for the carcass.

For the estimation of the percentages of fat and bone in the empty body the selected relations are:

Fat in empty body (Y); fat in 9–11 rib (X); relation 7, table 3,
$$Y = 0.68371\ X + 1.61401;\quad r_{xy} = +0.985;\quad S_{yx} = \pm 1.180.$$
Bone in empty body (Y); bone in 9–11 rib (X); relation 10, table 3,
$$Y = 0.51327\ X + 5.04322;\quad r_{xy} = +0.947;\quad S_{yx} = \pm 0.764.$$

The offal or rest of the empty body not accounted for in the definitions of the lean, fat, and bone in the empty body may be considered as the remainder necessary to make 100 percent. These relations are illustrated in figure 7.

Relations of prediction order exist in chemical composition between the empty body, carcass, and edible portion of the carcass and the wholesale rib cuts (table 4). It is reasonable to assume that similar correlations exist for the 9–11 rib cuts even though data did not exist for their valuation. It would seem practical in predicting the chemical composition of the three gross portions to make use of the mean ether extract-free composition. This procedure would only necessitate the estimation of the ether extract. Simple and partial correlations (table 7) show that the chemical composition on the ether extract-free basis is a function of age, having relatively small regression coefficients. There is little significance to the simple and partial correlation of the same composition with fatness (table 7). It is shown (fig. 4) that for the empty body the data for the 3 Jersey cows do not conform to that of the steers. The calculation of the statistical analysis for the 33 steers omitting the 3 cows shows an improvement in the correlation coefficients for the composition of the empty body correlated with age (table 8). The standard errors of estimate approximate the standard deviations (table 8).

Though the correlation coefficients of the ether extract-free composition and age are not extremely high it would seem desirable to make use of the function rather than to use the mean values. For practical use it is suggested that the ether extract-free composition calculated by the regression for ages of 10, 20, 30, 40, and 50 months be employed (table 9). When the ether extract-free composition is used as a practical constant it should be corrected for age by using the regression coefficient for the 33 Missouri steers for the empty body, and the regression coefficients for the entire 36 Missouri animals for the carcass and edible portion of the carcass. This selection is made according to the significance of the correlation coefficients.

If it is not practical to make a chemical analysis of the rib cut and only the physical composition is available the chemical composition may be approximated. This can be done by determining the percentage of fat in the empty body, carcass, and edible portion of the

carcass by the physical analysis as indicated and calculating the percentage of ether extract from the percentage of fat in these gross portions using relations 1, 2, and 3, table 5.

Empty body: Ether extract (Y), fat (X),
$Y = 1.06089 \ X + 3.11131$; $r_{xy} = +0.996$; $S_{yx} = \pm 0.954$.
Carcass: Ether extract (Y), fat (X),
$Y = 1.01885 \ X + 3.76939$; $r_{xy} = +0.994$; $S_{yx} = \pm 1.291$.
Edible portion of carcass: Ether extract (Y), fat (X),
$Y = 1.06206 \ X - 0.76254$; $r_{xy} = +0.994$; $S_{yx} = \pm 1.455$.
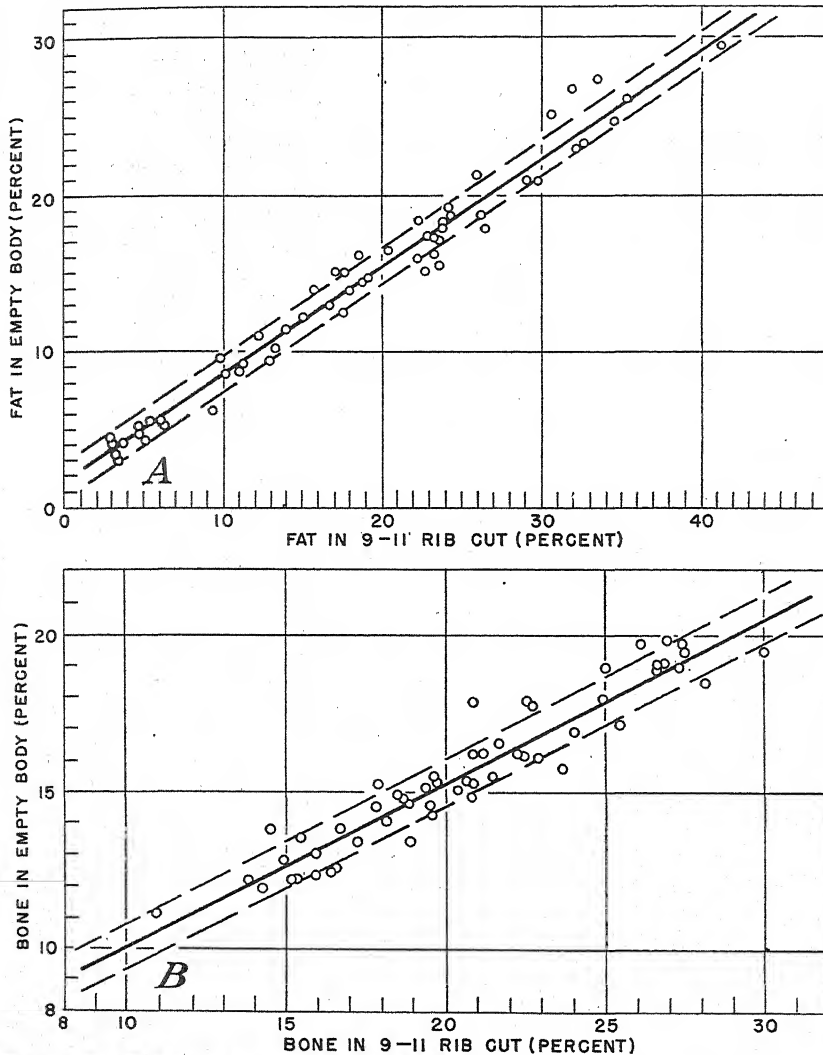
FIGURE 7.—Relations in percentage of fat (A) and bone (B) between the 9–11 rib cut and the empty body. Fat: $Y = 0.68371 \ X + 1.61401$; $r_{xy} = +0.985$; $S_{yx} = +1.180$. Bone: $Y = 0.51327 \ X + 5.04322$; $r_{xy} = +0.947$; $S_{yx} = \pm 0.764$.

However it should introduce less error to estimate the percentages of ether extract in the three gross portions from the determined ether extract in the rib cuts. This procedure is thought the most desirable. The relations suggested for use are:

Ether extract: Empty body (Y), 9–11 rib cut (X), relation 3, table 12,
$Y = 0.66234 \ X + 4.05099; \ r_{xy} = +0.983.$
Ether extract: Carcass (Y), 9–11 rib cut (X), relation 13, table 12,
$Y = 0.76289 \ X + 5.15647; \ r_{xy} = +0.983.$
Ether extract: Edible portion of the carcass (Y), edible portion of the 9–11 rib cut (X), relation 24, table 12,
$Y = 0.77976 \ X \ 3.40164; \ r_{xy} = +0.983.$

The ether extract having been estimated the moisture ash and crude protein can be estimated from the forecasted ether extract-free composition by using the values (table 9) for the age nearest to that of the animal under examination. As previously mentioned the constant extract-free composition values for the 33 steers should be used for the empty body and those for the entire 36 anmials should be used for the carcass and edible portion of the carcass.

The relations and the values in composition predicted from them must not be assumed to be perfect. The standard errors of estimate indicate variations inherent in the animal as well as those due to judgment in physical separation and to error in observation and analysis. Such inherent variation in the experimental animals and the constant presence of error in research methods and manipulations will naturally result in variability in the satisfaction of application of the experimental procedure proposed for the estimation of fatness and composition of the empty body and carcass of cattle. However it is more satisfactory than the procedures commonly used in experimental work and probably is the most accurate short of complete physical and chemical analysis. The proposed procedure should be applicable under a wide range of experimental facilities and conditions.

SUMMARY

The analytical data on 92 cattle have been studied statistically to determine the relations in physical and chemical composition that could be used for the prediction of the physical and chemical composition of the empty body, carcass, and edible portion of the carcass from the analysis of a rib cut. The wholesale rib, edible portion of the wholesale rib, the 9–11 rib, and the edible portion of the 9–11 rib cuts were studied as indicators of both physical and chemical composition.

The physical composition of the whole and edible portion of the wholesale and 9–11 rib cuts is highly correlated with the physical composition of the empty body, carcass, and edible portion of the carcass. This is especially true in the case of the percentage of fat.

The chemical composition of the whole and edible portion of the wholesale rib cut is highly correlated with the chemical composition of the empty body, carcass, and edible portion of the carcass.

There is a very high correlation between the percentages of fat (separable adipose tissue) and ether extract (true chemical fat) in the several portions of the animal studied.

The percentages of ether extract in the eye muscle, fat, and bone of the 9–11 rib are not considered satisfactory indices of fatness.

The dressing percentage is not a reliable indicator of fatness expressed either as fat or as ether extract.

The chemical composition on the ether extract-free basis is a function of age with small regression coefficient. For some purposes the mean might be considered a practical constant. However, for practical application the small variation with age should be recognized in using the composition on the ether extract-free basis as a means of prediction of the percentages of moisture, ash, and crude protein in the empty body, carcass, and edible portion of the carcass.

The percentage of ash in the edible portions may be predicted from the percentage of ether extract with nearly as satisfactory results as may be obtained by laboratory analysis.

The edible portion of the 9–11 rib cut was selected as an indicator of the physical composition of the edible portion of the carcass. For the physical composition of the carcass the percentage of bone is indicated by the percentage of bone in the 9–11 rib cut, and the percentages of lean and fat calculated from those estimated for the edible portion of the carcass. For the physical composition of the empty body the percentages of fat and bone are indicated by the percentages in the 9–11 rib cut, the weight of the lean taken as 1.0164 times the weight of lean in the carcass, and the "partial offal" taken as the remainder to make 100 percent.

The percentages of ether extract in the empty body and carcass may be indicated by the percentage of ether extract in the 9–11 rib cut and in the edible portion of the carcass from the percentage of ether extract in the edible portion of the 9–11 rib cut. Where chemical analysis is not practical, the percentages of ether extract may be estimated from the percentages of fat estimated or determined for the three gross portions. The correlation between the percentages of fat and ether extract in the same portion are extremely high. The percentages of moisture, ash, and crude protein in the gross portions may then be estimated from the mean ether extract-free composition corrected for variations due to age.

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BLISTER SPOT, A BACTERIAL DISEASE OF APPLE [1]

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INTRODUCTION

Blister spot, a relatively inconspicuous disease of apples, was first described briefly by Rose (*27*) [3] in 1916. In a later more detailed account (*28*) he named the causal organism *Pseudomonas papulans* (*Phytomonas papulans* (Rose) Bergey et al. (*4*)). Subsequently various reports of this and similar diseases appeared, but certain phases of the life history and physiology of the causal organism remained uninvestigated. Herein are reported the results of a detailed study of the physiology and pathogenicity of the blister spot organism, in comparison with other organisms, and of the pathological histology of the host. A description of the disease, which may be confused by the unaided eye with minute infections of the apple scab fungus, the known distribution of the disease, and notes on varietal susceptibility are also given.

REVIEW OF LITERATURE

After isolating the causal organism of the blister spot disease, Rose (*28*) reproduced the disease on a number of apple varieties by means of needle punctures or by hypodermic injections, but all attempts to reproduce it by inoculations on nonwounded fruits were unsuccessful. Rose described the morphological and physiological characters of the organism, which was a Gram-negative, white bacterium, producing green fluorescence in certain media. He also undertook a study of a disease that he called rough, or scurfy, bark disease of apple, the active stage of which is characterized by loosening and sloughing of the outer bark. He was successful in isolating a number of species of bacteria from the bark, and he concluded from certain physiological studies that these organisms were closely related to the blister spot organism (*28*). He obtained the typical blister spot disease of apples with two types of the scurfy bark organisms and thought that possibly all were varieties of one species; he suggested, however, that more work on the problem was necessary to clear up this point.

No further mention of the blister spot disease was found in the literature until 1924 when Rhoads (*25*), in an article on apple measles, stated that the role of *Phytomonas papulans* as a causal agent of scurfy bark, as previously suggested by Rose, was very doubtful. His

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attempts to isolate a causal organism from the pustular and scurfy types of measled apple bark were unsuccessful. He stated further that he was unable to find blister spot on apples in the orchards at Mountain Grove, Mo., as earlier described by Rose (*28*).

In 1927 Hopkins [4] published an account of a bacterial disease of apple trees occurring in the Elgin district of South Africa. When he compared the results of his physiological studies of the Elgin organism with two strains of *Phytomonas papulans* (one strain from bark, a slow liquefier of gelatin, and another, a rapid liquefier) and *Erwinia amylovora* (Burr.) Winslow et al., he concluded that the cultural characters of the Elgin organism and of the two strains of *Ph. papulans* clearly indicated that each was a distinct species. He made no attempt to obtain infection on fruits with the Elgin organism, nor is there any record of his having obtained infection on twigs or wood with *Ph. papulans*.

In 1931 Lacey and Dowson (*20*) described a disease of seedling apple trees characterized by cracks in the bark, horseshoe-shaped or circular in outline. Scattered among these were a number of small, raised swellings, or blisters. In most cases isolation from tissue under or within these blisters yielded almost pure cultures of a bacterial organism. When this organism, described as the Wisley strain, was inoculated into apple stems and twigs, lesions similar to those resulting from natural infections developed. In another series of experiments strains of this organism were inoculated on apples in various stages of development, from young to almost ripe fruit, but every attempt to induce infection failed. Inoculation of apple flowers also failed to produce infection. Immature apples and pears were inoculated and kept in moist chambers in the laboratory, but they, too, failed to develop the disease. Lacey and Dowson concluded that the organism which they described is identical with *Phytomonas papulans*, the cause of scurfy bark canker in America. They suggested that *Ph. papulans* should be considered a weak parasite, producing disease only when some physiological condition renders the trees susceptible to attack.

In 1932 Rosen (*29*) announced the discovery of a new pear and apple disease in North America simulating fire blight and having as its etiologic agent a pathogen belonging to the group typified by *Phytomonas barkeri* (*5*), *Ph. nectarophila* (*9*), and *Pseudomanas prunicola* (*37*).

In 1932 also Clara (*8*) published a brief account of a new pear disease caused by a green fluorescent bacterium. He noted that it was closely related to, but not identical with, *Phytomonas syringae* and he regarded it as a new species, which he named *Ph. utiformica*.

In 1934 Roberts (*26*) reported the isolation of a bacterium similar to *Phytomonas papulans* from early lesions associated with a disease which he had previously described as apple target canker. Inoculation with the organism on the variety Delicious produced lesions typical of the early stage of the disease. The results, however, were not conclusive, since similar lesions also appeared on the noninoculated checks. Roberts was of the opinion, however, that apple measles, target canker, and the rough bark disease might prove identical,

since their early symptoms were similar and what appeared to be the same bacterial species had been isolated from early stages of each.

Also in 1934 Dunegan (*12*) tested the susceptibility of the peach to artificial inoculation with *Phytomonas syringae, Ph. prunicola, Ph. mors-prunorum, Ph. papulans*, a bacterium from apple target canker, and an undetermined bacterial organism from a leaf spot of Italian prune. His studies showed that peach foliage was susceptible to these bacteria when they were actually introduced hypodermically into the tissues of leaves. There was a marked effect on the chloroplasts in the immediate vicinity of the point of inoculation and a stimulation in the formation of anthocyanin pigment in the surrounding regions.

In 1936 Wilson (*35*) reported the results of studies to establish the relation between a canker and blossom blast of pear and the bacterial canker of stone fruit trees. Besides the two strains of stone fruit organisms (*Phytomonas cerasi* and *Ph. cerasi* var. *prunicola*) and bacteria from pear limb cankers and blossom blast, his pathogenicity and cultural studies included the following organisms: *Ph. utiformica, Ph. papulans, Ph. citriputeale* (*Ph. citriputealis*), cultures obtained from pear blossoms in Arkansas and from limb canker of apple in California, and *Erwinia amylovora*. Wilson's inoculation and cultural tests supported the view that *Ph. utiformica, Ph. citriputeale*, and the bacterium isolated from pear blossoms in Arkansas were identical with the stone fruit organisms *Ph. cerasi* and *Ph. cerasi* var. *prunicola*. Wilson concluded that the bacterium isolated by Roberts from target cankers and provisionally designated as *Ph. papulans* was an unrelated species. From a later study of *Ph. syringae* and related organisms Wilson (*36*) concluded that *Ph. cerasi, Ph. utiformica*, and *Ph. prunicola* are synonymous with *Ph. syringae*.

THE BLISTER SPOT DISEASE

SYMPTOMS

Blister spot of apples is a relatively inconspicuous disease. Infections on fruits first noted in early June appear as darkened or water-soaked areas, often around lenticels. Later there are formed papillalike swellings or blisters, at first light in color but becoming brown or black. As pointed out by Rose (*28*), an occasional spot may develop into a larger irregularly lobed spot (fig. 1, *A* and *B*). In still later stages the epidermis over the blister dies and often cracks loose from the surrounding healthy tissue (fig. 2, *A*). This blister type with the accompanying darker color is the most conspicuous stage of the disease and by the unaided eye may be confused with minute infections of the apple scab fungus, *Venturia inaequalis* (Cke.) Wint. The disease at this stage is more conspicuous on yellow varieties such as Golden Delicious and Yellow Transparent than on highly colored ones such as King David, Jonathan, and Arkansas Black.

Close observations during the past 2 years and many isolations from suspected lesions have yielded no definite evidence that blister spot occurs naturally on leaves or twigs. A number of isolations from leaf spots of unknown origin yielded bacteria which resembled the blister spot organism in certain physiological and biochemical reactions. A discussion of this phase of the problem occurs on page 286.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Blister spot has been reported from Missouri,[5] Arkansas,[6] Pennsylvania,[7] Virginia,[8] Indiana,[9] and Illinois.[9] The original reports indicated that this disease caused considerable damage to susceptible varieties of apples at Mountain Grove, Mo., in 1916 and 1917. In 1938 the writer observed that 30 percent of the fruits in one of the experimental spray plots were affected with one or more blister spots. Zundel [10] reported in 1938 that in one orchard in Quincy, Franklin County, Pa., 20 percent of the apples had one or more blister spots. In 1939 blister spot was found at Mountain Grove on a greater number of varieties than in 1938, but the infection was less severe. Groves [11] reported it as prevalent in northern Virginia in 1939. In 1940 the disease was present in all the apple scab experimental spray plots at Mountain Grove; in certain plots 15 to 20 percent of the fruit showed infection. In 1940 the disease was seen for the first time at
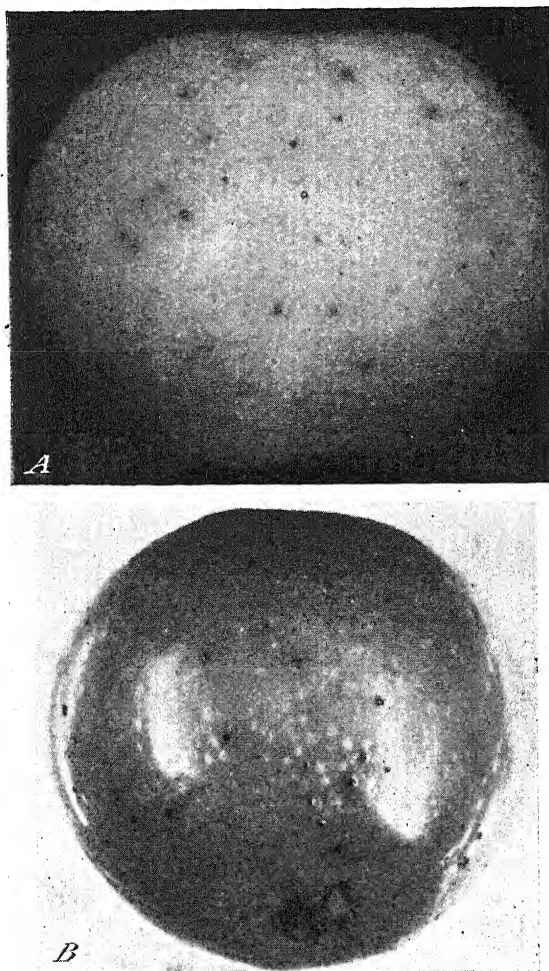
FIGURE 1.—*A*, Blister spot on Rome Beauty apple, × 1; *B*, young blister spot infections on Golden Delicious apple, × 1.

[5] SMITH, M. A. BLISTER SPOT ON ROME BEAUTY APPLES IN MISSOURI. U. S. Bur. Plant Indus., Plant Dis. Rptr. 22: 354. 1938. [Processed.]

[6] HUMPHREY, H. B., and WOOD, J. I. DISEASES OF PLANTS IN THE UNITED STATES IN 1933. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 86: 1–107, illus. 1935. [Processed.] (See p. 33, report on blister spot caused by *Bacterium papulans*, by J. C. Dunegan.)

[7] ZUNDEL, G. L. BLISTER SPOT OF APPLES IN PENNSYLVANIA. U. S. Bur. Plant Indus., Plant Dis. Rptr. 22: 377. 1938. [Processed.]

[8] NANCE, N. W. DISEASES OF PLANTS IN THE UNITED STATES IN 1939. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 128: 210–378, illus. 1941. [Processed.] (See p. 308, report on blister spot, caused by *Phytomonas papulans*, by A. B. Groves.)

[9] Correspondence.

[10] See footnote 7.

[11] See footnote 8.
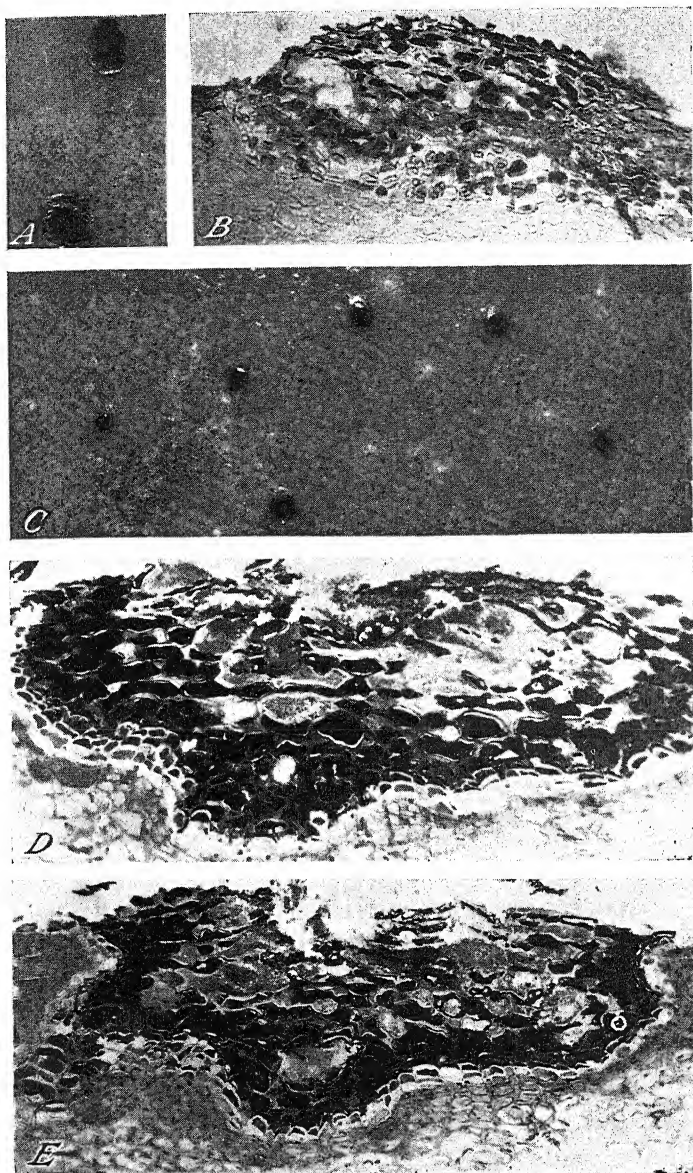
FIGURE 2.—*A*, Blister spot lesions, × 15. Note that the dead tissue has been separated from the surrounding tissue.   *B*, Cross section through a blister spot lesion, × 160. Note the raised pustular spot.   *C*, Recent infection of an apple by the blister spot pathogen, × 5.5.   *D* and *E*, Cross sections through blister spot lesions showing masses of bacteria throughout the host tissues (*D*) and between the diseased tissue and the phellogen layer (*E*), × 200.

Elsberry, Mo., and in other orchards in the central and southeastern parts of the State.

Blister spot is relatively inconspicuous because of the smallness of the usual type of lesions. In years of light infection most of the apples on a tree may be entirely free of the disease and others may have only a few spots. When infections are more prevalent, the lesions may vary from few to many per fruit. Lesions on highly colored fruit may easily be overlooked.

During the past 3 years the writer has not observed any instance of commercially important damage to the crop. The apparent increase in the number of varieties affected by this disease at Mountain Grove during the past 2 years suggests a possible increase in severity of infection at some future time if conditions should be particularly favorable. At present the disease is considered of minor economic importance.

VARIETIES [12] NATURALLY INFECTED

Blister spot has been found under natural conditions only on apple fruits. In 1915 the disease was reported on Hawley, Ishewood, Melon, and Norfolk Beauty. In 1916 it was found on Benoni, Blue Pearmain, Duling, Early Ripe, Hawley, Higginbotham, Isham, Ishewood, Jonathan, Klondike, Lansingburg, Melon, Norfolk Beauty, Red Astrachan, Rock Pippin, White Pippin, Yellow Transparent, and Victuals and Drink. During the past 2 years the writer has observed blister spot on the following: Arkansas Black, Benoni, Black Twig, Carson, Colton, Delicious, Golden Delicious, Jonathan, King David, Liveland, Maiden Blush, Red Astrachan, Rome Beauty, Stayman, Turley, Twenty Ounce, White Winter Pearmain, and Willow Twig.

PATHOLOGICAL ANATOMY OF AFFECTED FRUIT

Small portions of fruit containing blister spots were killed and fixed in a mixture of formalin, acetic acid, and alcohol, embedded, and sectioned in the usual manner. The ethyl alcohol-xylol-paraffin method and the n-butyl alcohol-paraffin method of Zirkle (*38*) were used. Occasionally freehand sections of fresh material were made.

Microtome sections 6μ to 10μ thick were stained with basic fuchsin, carbol fuchsin, or gentian violet, with orange G as a counter stain. The thionin-orange-G staining technique, as reported by Stoughton (*33*), was used also and proved to be the most satisfactory for photomicrographs.

Cross sections prepared from blister spots show papillalike swellings on the surface (fig. 2, *B*). The organism gains entry through wounds or lenticels. On recently infected fruits the disease appears macroscopically as spots with water-soaked margins (fig. 2, *C*). Freehand sections of spots collected in the early summer (June 15) often show the cuticle immediately surrounding the blister spot to be unbroken. Immediately beneath the cuticle the host cells are dead.

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[12] The nomenclature for the apple varieties listed in this paper is largely that of Beach et al. (*3*), Hedrick (*16*), and Downing (*10*). A few varieties not listed in these publications are American varieties that are not often grown commercially. Hedrick's nomenclature is followed also for some of the other fruit varieties.

Serial cross sections show the diseased area to extend often as much as 0.4 mm. below the cuticle, but as a rule the lesions are not so deep. Stained sections show the bacteria to be present intercellularly throughout the region of the papule.   Immediately beneath the diseased area a phellogen layer from 3 to 5 cells in thickness develops (fig. 2, *D*). In some sections the invading organism is seen to have broken through this layer, but invariably it is again walled off by a layer of phellogen which effectively bars it from further extension.
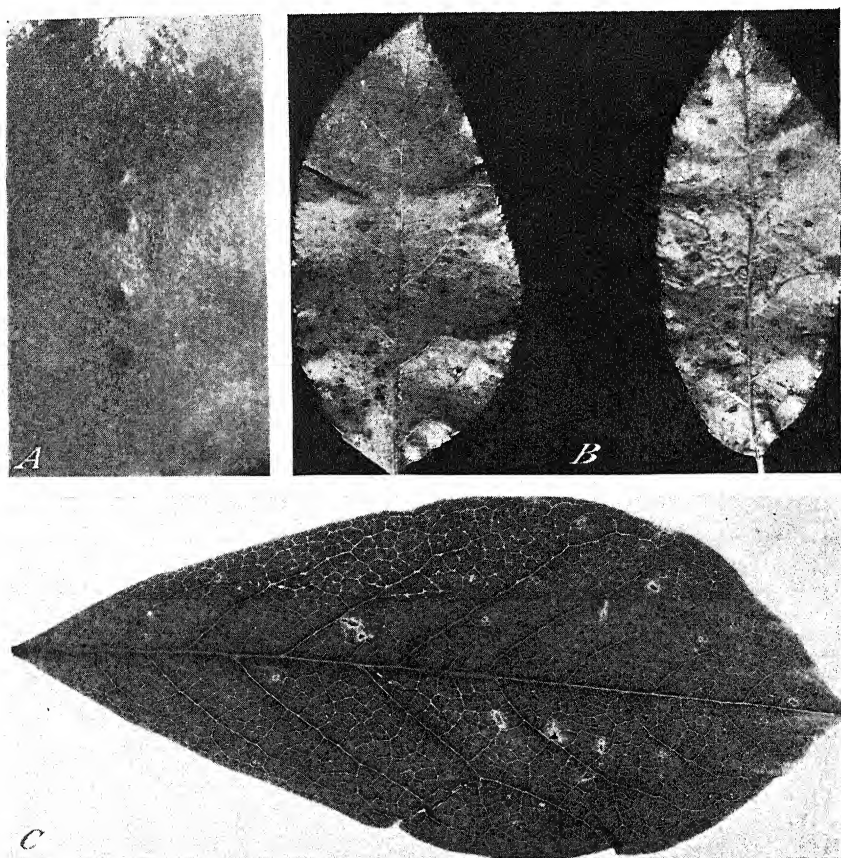
FIGURE 3.—*A*, Artificial infection (needle-puncture inoculation) of Red Astrachan apple by the blister spot organism, × about 2.   *B*, Bacterial spot disease on Rome Beauty apple leaves, × about 1.   *C*, Bacterial spot disease on leaf of × *Magnolia soulangeana*, × about 1.

Pockets composed of phellogen layers of cells surrounding masses of bacteria are found throughout many of the lesions; it is presumed that these formations are due to the counteraction of the host against bacterial invasion (fig. 2, *E*).   Sections of artificially infected tissue show characteristics similar to those of naturally infected tissue. However, it has been observed that when fruits have been artificially inoculated in the laboratory, a definite swelling occurs in the immediate vicinity of the needle puncture, but blisters such as one observes

in the naturally infected fruit are not formed (fig. 3, *A*). In later stages of natural lesions the epidermis over the blister becomes black and dies. As a rule when this occurs, the injured epidermis cracks loose from the surrounding healthy portion (fig. 2, *A*). Where there is no disruption of the epidermis the blister spot may become larger and the lesion may extend outward in various directions, but this condition is rarely observed. Most of the infections in late summer are of the typical blister type.
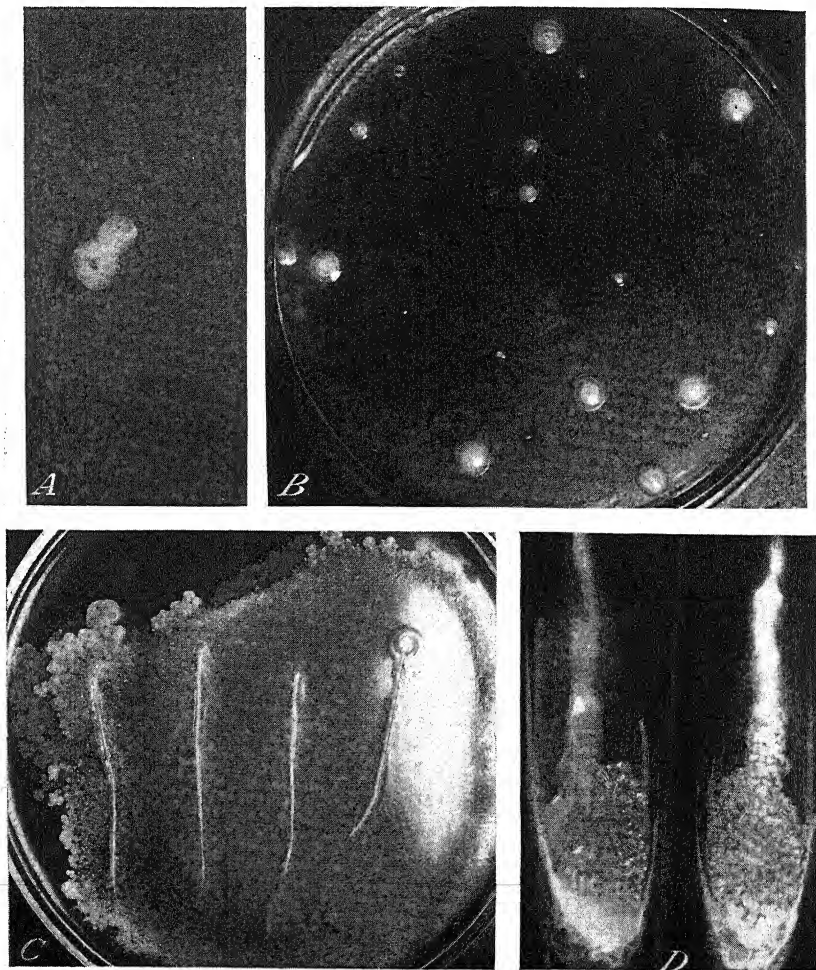
FIGURE 4.—*A*, Bacteria oozing from blister spot planted on beef-infusion agar, × 1. *B–D*, Typical growth of the blister spot organism, × 1: *B*, After 5 days on beef-infusion agar; *C*, after 4 days on Patel's medium; *D*, after 48 hours on beef-infusion-glycerin agar.

SOURCE OF ORGANISMS STUDIED

The blister spot organism is easily isolated from infected apples by sterilizing the surface with 80 percent alcohol, removing a papilla

with a sterilized scalpel, dropping it into a tube of sterile water, and making dilution plates after the bacteria have diffused into the water. Colonies appear in 36 to 48 hours, usually in pure culture (fig. 4, *B*). Isolations may also be made by placing the sterilized papillae directly in poured agar plates (fig. 4, *A*).

Isolations from blister spots have been made from the first appearance of infection in June until mid-September. The percentage of successful isolations from old spots is materially decreased, particularly from those from which the blister has sloughed off, leaving no protection against desiccation. The occurrence of small and shallow blisters seems to indicate that the blister spot organism is a relatively weak parasite.

Rose (*28*) isolated a number of organisms from rough, or scurfy, apple bark. These apparently belonged to the green fluorescent group of bacteria. One of them appeared to have all the cultural characteristics of the blister spot pathogen and when inoculated into healthy apples produced typical blister spots. The writer has made a careful search during the past 2 years for this scurfy bark manifestation. Isolations have been made from cankers of many types, but none has yielded an organism resembling that originally mentioned by Rose.

During the past 2 years a leaf spot characterized by round to irregular purplish areas, located generally in the vicinity of the midvein or veinlets, has been observed often on the leaves of the Rome Beauty apple (fig. 3, *B*). At first this type of spotting was confused with that produced by early infections of *Venturia inaequalis*, which it somewhat resembles. Further examinations of small sections cut in water drops showed masses of bacteria oozing from them in a high percentage of cases. Isolations from such spots often yielded pure cultures of a bacterium which proved to belong to the green fluorescent group. Inasmuch as the blister spot organism had not been reported previously as occurring on apple foliage, it seemed important to make physiological and pathological tests to determine whether any relationship might exist between the blister spot organism and this bacterium from an undescribed apple leaf spot.

During May 1940 the writer also observed a leaf spot on the foliage of × *Magnolia soulangeana* Soul. The spots were round to irregular, water-soaked, ranging in size from 1 to 3 mm., and most apparent on the upper leaf surfaces (fig. 3, *C*). Large numbers of bacteria could be seen oozing from sections of tissue placed in water. A high percentage of isolation cultures from such material yielded a bacterium which later produced a green fluorescence in the beef-infusion agar used as the culture medium. Since this organism from magnolia foliage belongs to the green fluorescent group of bacteria, it seemed important to study it.

The writer's investigations, intended to supply more complete information about the essential physiological characteristics of the blister spot organism than is furnished in the original characterization, included 18 blister spot isolates, 3 isolates of *Phytomonas syringae* (Van Hall) Bergey et al., 2 isolates of the target canker organism provisionally thought to be *Ph. papulans*, and 1 isolate each of the bacteria from the apple and magnolia leaf spots mentioned herein.

Of the 18 isolates of the blister spot organism, 2 were obtained in 1938, 13 in 1939, and 3 in 1940; 1 was from Arkansas and the rest from 4 localities in Missouri. All were originally isolated from apple fruits of several varieties; 7 had been reisolated after artificial inoculation into apple, plum, tomato, or lemon. Cultures of *Phytomonas syringae* from lilac (*Syringa vulgaris* L.), designated as SI-1 and SH-2 and obtained from Mary K. Bryan, formerly of this Division, had been received originally from Illinois and the Netherlands, respectively; the third isolate designated NY (New York strain) was obtained from W. H. Burkholder, Ithaca, N. Y. Target canker isolates T-62 and T-64 from apple were supplied by J. W. Roberts. The magnolia leaf spot organism was designated MG-19, and the one from Rome Beauty apple leaves was called A-20.

MORPHOLOGY AND STAINING REACTIONS OF BLISTER SPOT AND RELATED ORGANISMS

For uniform determination of form and size of the various isolates smears were made from cultures in beef-extract peptone broth of pH 6.8 which had been incubated for 24 hours at 25° C. Maneval's (*22*) method of negative staining was employed in an attempt to avoid distortion in shape or in size such as might have resulted from the application of a direct stain. In staining flagella the Casares-Gil method, as published by Plimmer and Paine (*24*) and given by the Society of American Bacteriologists (*32*), was employed. The method of Maneval (*21*) was also used. For Gram-staining Hucker and Conn's modification of the ammonium-oxalate-crystal-violet method (*17, 18*) and Kopeloff and Beerman's modification (*19*) were used. The Ziehl-Neelsen method of acidfast staining was used. The Anthony method (*1*) of capsule staining was used. In table 1 the blister spot organism is compared with the other organisms studied.

PATHOGENICITY OF BLISTER SPOT AND RELATED ORGANISMS

The pathogenicity of the blister spot organism was first demonstrated by Rose (*27*) in 1916. Using subcultures from single colonies, he reproduced the disease on six varieties of apples. The fruits were inoculated while on the trees and were bagged after inoculation. The incubation period averaged 14 days, but some fruits failed to show signs of the disease until after 18 to 25 days. Some strains of the organism infected all the varieties tested and others only one or two. The pathogenicity studies carried on by the writer in 1939 and 1940 are described herein.

FIELD INOCULATIONS

In 1939 inoculations of apple fruits, twigs, and leaves were made by four methods: (*a*) By means of needle punctures, a water suspension of the organism being introduced into a shallow puncture under the epidermis; (*b*) by hypodermic injection of a water suspension of the organism under the epidermis; (*c*) by needle puncture of fruits which had first been atomized with a water suspension of the organism; and (*d*) by atomizing a culture of the organism in water suspension on the surface of nonwounded fruits or leaves. In the needle-puncture inoculation three wounds were made in each twig or fruit. Inoculation of twigs was made by piercing the bark tangentially and then

TABLE 1.—*Comparison of morphology and staining reactions of blister spot and related organisms*

Organism and strain	General description	Size	Capsules demonstrated	Spores	Polar flagella	Gram-negative	Effective stains	Acidfast
Blister spot	Motile rods with rounded ends, usually occurring singly; occasionally in pairs or short chains.	μ 0.8–2.5×.8	No	No	1 to several	Yes	Carbol fuchsin, crystal violet; methylene blue.	No.
Phytomonas syringae	do	1.3–1.9×.5	No	No	do	Yes	Crystal violet; methylene blue.	No.
Target canker isolate (T–62).	Short rods with rounded ends, usually occurring singly.	1.6–1.9×.3	No	No	Nonmotile	Yes	do	No.
Target canker isolate (T–64).	Short rods with rounded ends, occurring singly or in pairs; sometimes in chains.	1.5–2.6×.5	No	No	Not demonstrated	Yes	do	No.
Magnolia isolate (MG–19).	Short motile rods with rounded ends, usually occurring singly.	1.2–1.7×.6	No	No	1 to several	Yes	do	No.
Isolate from apple leaf spot (A–20).	Short motile rods, usually occurring singly; sometimes in pairs or chains.	1.5–2.7×.5	No	No	do	Yes	do	No.

injecting a water suspension of the organism into the opening in the bark. Inoculation of leaves was made by spraying noninjured leaf surfaces with a water suspension of the organism or by scratching either the upper or the lower leaf surface with a hypodermic needle and at the same time introducing a water suspension of the organism. After fruits, either wounded or nonwounded, were inoculated, they were wrapped in a thin layer of moist cotton and then covered with a layer of Parafilm, which was allowed to remain for 24 hours after the inoculation. The points at which the needle punctures were made in twigs were covered by a layer of Parafilm; this was sufficiently elastic to allow for growth expansion and at the same time to give an adequate covering. The inoculated leaves were enclosed in a cellophane bag for a period of 24 hours. In all cases an adequate number of sterile-water (check) inoculations were made in comparable fruit, leaves, or twigs of the same trees.

In 1940 the methods were the same, except that method *c* was not used.

In 1939 inoculations were begun in May and continued at intervals until October; inoculations were begun again in April 1940 and continued until September. The results are summarized in table 2.

TABLE 2.—*Summary of field inoculations with blister spot and other organisms, Mountain Grove, Mo., 1939 and 1940*

Organism and strain	Year and host	Varieties inoculated	Part inoculated	Inoculations made	Method of inoculation [1]	Average period in which infection occurred	Varieties infected	Inoculations resulting in infection (all varieties inoculated)
		Number		*Number*		*Days*	*Number*	*Percent*
Blister spot:	*1939*							
	Apple	8	Fruit	140	a	21	5	19
	---do	2	---do	30	c	26	2	23
	---do	3	---do	70	d	26	3	4
	---do	1	Leaf	10	b			0
	---do	2	Twig	30	a	36	2	40
	Cherry	4	---do	60	a	28	2	10
No. 1	Pear	1	---do	15	a	28	1	33
	---do	1	---do	15	b			0
	Peach	1	---do	15	a			0
	---do	1	Leaf	15	b	41	1	13
	1940							
	Lilac	1	Shoot	15	a	12	1	40
	Apple	1	Leaf	15	b			0
	1939							
	Apple	9	Fruit	165	a	19	9	57
	---do	1	---do	15	c	25	1	13
	---do	1	---do	30	d	14	1	13
No. 2	---do	3	Leaf	75	b	14	3	32
	---do	2	---do	30	d			0
	---do	2	Twig	45	a	41	2	16
	1940							
No. 7	Apple	1	Fruit	20	a	22	1	80
	Apple	1	Leaf	20	d			0
	Apple	1	Fruit	15	d	21	1	73
	---do	1	---do	15	a	23	1	26
No. 9a	Apple	1	Twig	15	a	32	1	13
	Lilac	1	Shoot	15	a	10	1	60
	Pear	1	Twig	15	a	26	1	33
No. 10	Apple	1	---do	20	a	22	1	45
	Lilac	1	Shoot	15	a	11	1	26
	Apple	1	Twig	15	a			0
No. 12	---do	1	Fruit	15	d	20	1	40
	Plum	1	Twig	15	a	21	1	13
No. 13	Apple	1	Fruit	15	a	23	1	13
	Lilac	1	Shoot	15	d			0

[1] See p. 278 for description of methods of inoculation.

TABLE 2.—*Summary of field inoculations with blister spot and other organisms, Mountain Grove, Mo., 1939 and 1940*—Continued

Organism and strain	Year and host	Varieties inoculated	Part inoculated	Inoculations made	Method of inoculation	Average period in which infection occurred	Varieties infected	Inoculations resulting in infection (all varieties inoculated)
	1939	*Number*		*Number*		*Days*	*Number*	*Percent*
	Apple	1	Leaf	15	a	----	----	0
	do	1	Fruit	20	d	20	1	20
	do	1	do	15	a	21	1	66
	do	1	Leaf	15	b	22	1	46
No. 14	Lilac	1	do	15	b	11	1	46
	Magnolia	1	do	20	b	7	1	50
	do	1	do	20	d	----	----	0
	do	1	Petiole	20	a	6	1	60
	Plum	1	Twig	15	a	----	----	0
No. 15	Apple	1	do	15	a	----	----	0
	Apple	2	do	30	a	----	----	0
No. 16	do	1	Leaf	20	b	23	1	30
	Lilac	1	Shoot	15	a	12	1	46
No. 17	Apple	1	Fruit	15	a	20	1	33
No. 18	Apple	1	Twig	15	a	26	1	40
	do	1	Fruit	15	a	----	----	0
Phytomonas syringae:	*1939*							
	Apple	7	do	70	a	----	----	0
	do	2	do	20	d	----	----	0
SI-1	do	1	Leaf	20	b	----	----	0
	do	1	do	10	d	----	----	0
	do	1	Twig	10	a	----	----	0
	Cherry	4	do	40	a	----	----	0
	1940							
	Apple	2	Fruit	30	a	18	1	20
	do	2	do	30	d	----	----	0
NY	do	1	Twig	15	a	35	1	13
	Lilac	1	Leaf	15	b	15	1	80
	do	1	Petiole	15	b	15	1	80
	do	1	Shoot	15	a	15	1	66
	Plum	1	Twig	15	a	32	1	33
Target canker:								
T-62	Apple	2	Fruit	60	a			0
	do	2	do	30	d			0
	do	2	Twig	60	a			0
T-64	Apple	2	Fruit	50	a			0
	do	2	do	35	d			0
	do	2	Twig	40	a			0
	Apple	3	Fruit	60	a	19	2	27
	do	2	do	45	d	----	----	0
	do	1	Twig	15	a	27	1	33
	Lilac	1	Leaf	10	a	11	1	60
	do	1	do	10	d	----	----	0
Magnolia (MG-19)	do	1	Petiole	10	b	13	1	40
	do	1	Shoot	10	a	8	1	70
	Magnolia	1	Leaf	10	b	14	1	90
	do	1	do	10	d	----	----	0
	do	1	Petiole	10	a	14	1	70
	Plum	1	Twig	15	----	24	1	20
	Apple	1	Fruit	20	a			0
	do	1	do	25	d			0
Apple leaf (A-20)	do	1	Leaf	15	a			0
	do	4	do	45	b			0
	do	1	Twig	15	a			0
	Plum	1	do	10	a			0

In 1939 infection was obtained on Rome Beauty, Willow Twig, Jonathan, Stayman, Delicious, Red Astrachan, Red June, Yellow Transparent, Carson, Colton, Early Harvest, and Liveland apples. Infection was not obtained on King David, Winesap, and Arkansas Black. The infection obtained on peach leaves confirmed the results of Dunegan (*12*), who found peach susceptible to blister spot and other bacteria actually introduced into leaves and twigs. Unlike the blister spot organism, *Phytomonas syringae* SI-1 did not infect any of the varieties of apple or cherry.

In 1940 infection was obtained on Rome Beauty, Golden Delicious, Delicious, and Willow Twig apples. Infection was not obtained on Black Twig and Stayman. Apple twigs (fig. 5, *C*) and fruit were also infected by the New York strain of *Phytomonas syringae* and by the
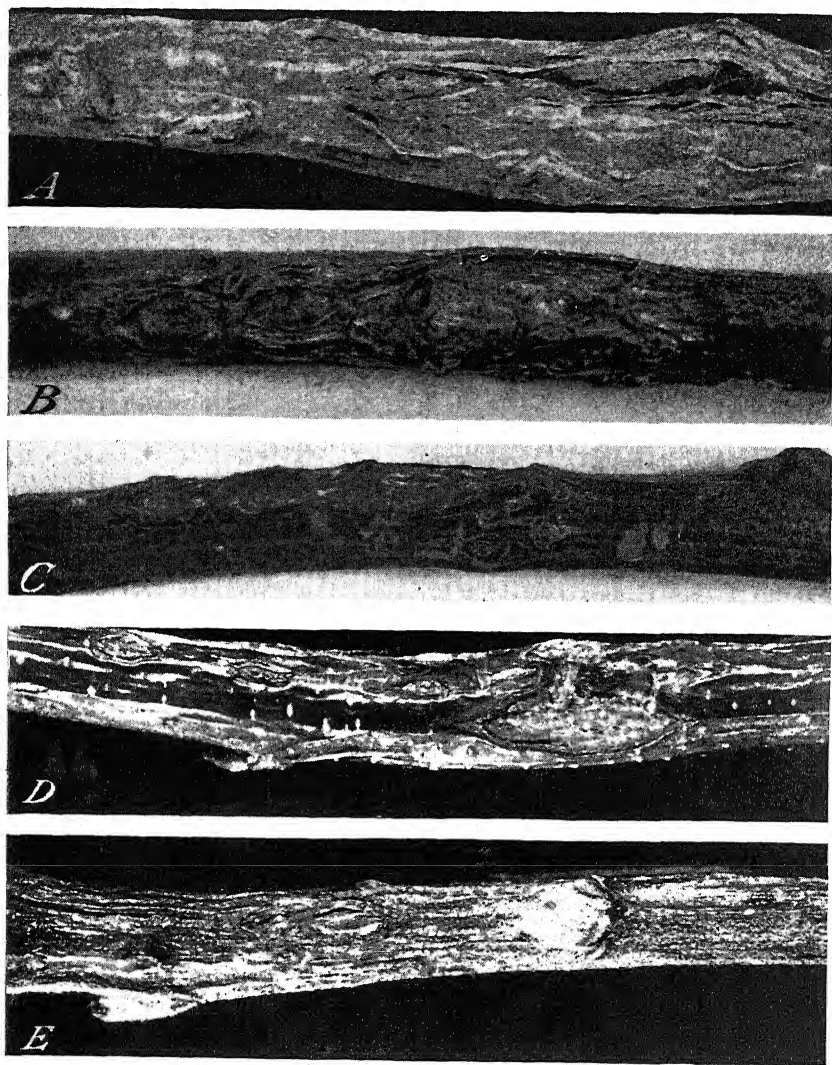
FIGURE 5.—*A* and *B*, Twigs artificially infected with the blister spot organism, × 2: *A*, President plum 25 days after inoculation; *B*, Willow Twig apple 26 days after inoculation. *C* and *D*, Artificial infection of twigs with NY strain of *Phytomonas syringae: C*, Rome Beauty apple, × 2; *D*, President plum, × about 3. *E*, Check puncture on President plum, × about 3.

magnolia isolate. Lilac and plum were both infected by blister spot, *Ph. syringae*, and magnolia isolates; magnolia also was infected by blister spot and magnolia isolates (see fig. 5, *A* and *D*).

When lilac leaves were inoculated with the blister spot organism by scratching the surfaces with a hypodermic needle containing a water suspension of the organism, the infection appeared as greenish-yellow lines along the path of the needle scratches. A water-soaked area extending on either side of the scratch was often visible on the fourth or fifth day after inoculation. No infection was obtained when nonwounded leaf surfaces were sprayed with a water suspension of the organism. Infection on lilac shoots was localized, the most pronounced type of lesion being characterized by slightly raised, irregular, purplish spots, often not more than 3 to 4 mm. in diameter. Some spots had water-soaked areas around them. Pure culture re-isolations of the organisms from these lesions were readily made. There was never any indication of wilting or extensive vascular infection such as has been shown by Bryan (6) to occur when lilac is inoculated with *Phytomonas syringae*.

Rose (28) stated that some strains of *Phytomonas papulans* were infectious to all varieties of apples tested and others to only one or two, and suggested that further work was necessary. In the present tests no clear-cut strain differences were demonstrated.

The highest percentages of infection with the blister spot organism were obtained during May and June and the lowest during July, August, and September; no infections were obtained from inoculations made in April or October. The average incubation period on fruit inoculated with the blister spot organism was 21 days. In general, as expected, the fewest infections were obtained in nonwounded tissue. The highest percentage of infection on twigs was obtained on those that were growing rapidly.

The lesions produced on apple fruits by wound inoculations with *Phytomonas syringae* were slightly irregular, brown to black, water-soaked spots. There was no evidence of the production of a papilla, which is characteristic of the type of infection produced by the blister spot organism. Artificial inoculations of apple fruits performed under the same conditions with the blister spot organism resulted in infections showing characteristic papillae. No infection was obtained with *Ph. syringae* when nonwound inoculations were made on fruits.

INOCULATION OF IMMATURE APPLES AND STONE FRUITS

On June 6, 1939, immature apple, plum, cherry, and peach fruits were removed from trees and inoculated with a water suspension of a 24-hour-old culture of the blister spot organism by making four needle punctures through drops on each fruit. Checks consisted of punctures made through sterile-water drops. The fruits were held in moist chambers at 25° C. for 8 days. Doubtful infections were checked by culturing on beef-infusion-agar plates and any suspected organisms by further inoculations of fruits.

Successful inoculations, varying from 80 to 100 percent, were obtained on 7 apple varieties. These in order of susceptibility were White Pippin, Grimes, Skelton, Ada Red, Polly Eads, Twenty Ounce, and Grays Red. Smaller percentages of infection occurred on 17 apple varieties, as follows: Fanny, Ishewood, Colton, Lansingburg, Summer Champion, Benoni, Victuals and Drink, Early Ripe, Carson, Wealthy, Early Harvest, Sops of Wine, Red June, White Winter Pearmain, Lowell, Carson, and Red Astrachan. Inoculations failed

on 9 varieties (Yellow Transparent, Liveland, Early McIntosh, Duchess, Maiden Blush, Pennock, Henry Clay, Hopa Crab, and Whitney Crab). Indian Blood plum and Yellow Glass cherry were successfully inoculated. Inoculations of Gold cherry and Elberta peach were not successful.

This experiment shows that immature apple (fig. 3, *a*), plum (fig. 6), and cherry fruits may be removed from the trees and successfully inoculated in moist chambers. Since the incubation period is relatively short (5 to 8 days), the method is of value for quick testing of the pathogenicity of the blister spot organism.

During the 1939 season two attempts were made to induce infection in moist chambers of nonwounded immature fruits of 24 varieties of apples. Ten fruits of each variety were atomized with a water suspension of a 24-hour-old culture of the blister spot organism and held in moist chambers at a temperature of 26° C. Nonwounded fruits atomized with sterile water served as checks. In no instance was there definite evidence that infection had occurred during a 12-day period.

FIGURE 6.—Artificial infection of Indian Blood plum with the blister spot organism, × about 5.

INOCULATION OF TOMATOES

A water suspension of the blister spot organism was introduced by means of needles into 12 mature and 12 immature tomato fruits in moist chambers. Within 3 days water-soaked areas appeared around most of the points of inoculation on the immature fruits, but not on the similarly inoculated mature fruits. When final observations were made 1 week later, no infection was evident on the mature, inoculated fruits or on the check fruits. The diseased areas which occurred at most of the points of inoculation on the immature fruits consisted of black, water-soaked, slightly raised lesions about 2 to 5 mm. in diameter. Reisolations of the organism from such lesions were readily made. Smith and Fawcett (*31*) obtained similar results with the citrus blast organism, *Phytomonas citriputeale*, when they inoculated fruits of tomato a short time before the fruits reddened. Their report does not show that mature tomato fruits were inoculated.

INOCULATION OF LEMONS AND ORANGES

Smith and Fawcett (*31*), Rosen and Bleecker (*30*), and others have tested the infectivity of certain fluorescent bacteria, such as

Phytomonas syringae, *Ph. cerasi*, and *Ph. citriputeale*, by inoculating lemons or oranges and noting the size of lesions produced on them.

The writer has made many inoculations of lemon and orange fruits with isolates of the blister spot organism, *Phytomonas syringae*, and the target canker, magnolia, and apple leaf spot organisms. The *Ph. syringae* isolates have, in most instances, produced a characteristic black pitting on these hosts, and reisolations were readily obtained.

The blister spot isolates have never produced this typical black pitting. However, 8 days after inoculation with this organism the tissue immediately surrounding the needle puncture becomes sunken and somewhat water-soaked in appearance. After 10 days there may be a light-brown discoloration at the point of inoculation and the tissue 1 to 2 mm. beneath the skin in the infected areas has a glassy, water-soaked appearance. A microscopic examination of this area reveals the presence of large numbers of bacteria, and in most instances reisolations yield pure cultures of the blister spot organism.

The magnolia isolate when introduced into lemons and oranges has produced the typical black pitting characteristic of *Phytomonas syringae* inoculations. Successful inoculations of lemons and oranges with the two target canker isolates and the apple leaf spot isolate have not been obtained.

OVERWINTERING OF THE BLISTER SPOT ORGANISM

During the course of these studies numerous isolations from twig and wood cankers and leaves of the previous year have been attempted in an effort to discover how the blister spot pathogen survives the winter. None of these has proved successful. Inasmuch as apple fruit infections are the only known natural manifestation of the disease, it seemed important to study the overwintering of infected fruit.

In October 1939, Rome Beauty apple fruits affected by the blister spot disease were placed on the ground in the orchard and one-fourth-inch mesh wire screen was placed over them as a partial protection against rodents. Beginning October 16 and at approximately 1-month intervals throughout the fall and winter of 1939–40, isolations were attempted by removing the blisters from the apples with aseptic precautions, crushing them in tubes of sterile water, and culturing them on plates of Patel's sodium taurocholate medium (*23*), which was found to be the most satisfactory for this purpose. Table 3 records the results of these studies.

In this experiment the blister spot pathogen remained viable from October 16 to March 15, but no growth occurred in the cultures made in April and May. Fewer blister spots were plated in the winter and spring months because gradual disintegration of the fruits made it difficult to select suitable material.

The apparent failure of the pathogen to remain viable after March 15 seems to indicate that blister spots on overwintering fruits do not constitute a source of infection in the spring. It is obvious that the pathogen would have to survive at least until May 1 to initiate infection on young fruits of the next crop. As these results apply to one season only, they do not wholly preclude the possibility of overwintering of the organism on the fruit.

TABLE 3.—*Overwintering of blister spot pathogen on apple fruits*

Date of culture	Fruits	Blister spots	
		Cultured	Viable
1939	*Number*	*Number*	*Number*
Oct. 16	24	22	14
Nov. 15	17	14	6
Dec. 14	12	15	6
1940			
Jan. 17	6	12	4
Feb. 15	5	5	1
Mar. 15	2	6	1
Apr. 18	1	3	0
May 15	1	1	0

PHYSIOLOGY OF BLISTER SPOT AND RELATED ORGANISMS

In studies of the cultural characters and biochemical reactions of the various isolates, the methods used were essentially those recommended by the Society of American Bacteriologists (*32*). The hydrogen-ion concentration of all the media used was determined by the colorimetric and quinhydrone-electrode methods. All the organisms were cultured in beef-extract-peptone broth (pH 6.8 to 7.0) for 12 hours before being transferred to the media used in the studies. Unless otherwise stated, all cultures were incubated at 25° C. The physiological studies were based on 18 isolates of the blister spot organism, 3 isolates of *Phytomonas syringae*, 2 isolates of the target canker organism, and 1 each of the magnolia and the apple leaf spot organisms.

GROWTH ON VARIOUS MEDIA

BEEF-INFUSION-AGAR SLANTS.—On beef-infusion agar adjusted to a pH of 6.8, the growth of the blister spot organism was visible after 24 hours as a grayish-white, filiform line following the path of the needle. After 3 days the growth was abundant, filiform, glistening, butyrous, and white. After 10 days the medium was greened. It was observed occasionally that certain of the blister spot isolates which had been in culture for a long time had apparently lost their ability to produce pigment. This feature of the fluorescent plant pathogens was mentioned by Burkholder (*7*). In some isolates the lower end of the slant assumed a pink tinge after 3 weeks.

The growth of *Phytomonas syringae* and of the magnolia isolate was flatter than that of the blister spot organism, but otherwise they exhibited the same characteristics. Target canker isolate T-64 was characterized by abundant, filiform, glistening, grayish-white, somewhat slimy growth with a tendency to darken after 8 days. Greening of the medium was more prompt than with the blister spot organism or *Ph. syringae*. With target canker isolate T-62, the growth was scant, filiform, viscid, and flat, and the medium was not greened. The apple leaf isolate was characterized by abundant, filiform, slimy, glistening, grayish-white growth becoming grayish brown after 10 days. The medium was greened.

BEEF-INFUSION BROTH.—In beef-infusion broth (pH 6.9) the blister spot organism, *Phytomonas syringae*, the magnolia isolate, and target canker isolate T-64 produced abundant clouding in 24 hours. With the blister spot organism green fluorescence began in 3 days, starting at the top. The pellicle was of delicate structure and broke easily upon jarring. The sediment was flaky, and there was no odor. *Ph. syringae* isolates were indistinguishable from the blister spot organism except that there was slightly less clouding. Growth of target canker isolate T-62 was scant, and no pellicle was formed. There was no sediment and no greening of the medium. With the apple leaf isolate growth was abundant, a slight pellicle was formed, and the medium was greened.

BEEF-EXTRACT BROTH.—The growth of the blister spot organism, *Phytomonas syringae*, the magnolia isolate, target canker isolate T-64, and the apple leaf

isolate was the same as in beef-infusion broth except that the pellicles formed were less stable and there was no greening of the medium. Target canker isolate T–62 did not form a pellicle, and there was no greening of the medium.

BEEF-INFUSION-AGAR PLATES.—Colonies of the blister spot organism were visible in dilution plates after 48 hours. They were round, smooth, entire, white, convex, and opalescent; some isolates became concentrically ringed after 5 days (fig. 4, *B*). The medium was greened. Illinois, Netherlands, and New York strains of *Phytomonas syringae* closely resembled the blister spot organism during the first 48 hours of their growth. As a rule, the colonies of *Ph. syringae* were smaller than those of blister spot. The Netherlands strain retained its smooth surface at all times. The Illinois strain became cross-hatched in 4 days. As this strain increased in age, the wrinkled appearance grew more pronounced. The New York strain was indistinguishable from the Netherlands strain. The colonies were smooth with no cross hatching. The colonies of the magnolia isolate were round, slightly raised, and opalescent and had rough edges. The medium was greened. Colonies of target canker isolate T–64 were round to irregular, cream-colored, and convex; the medium was greened. Colonies of target canker isolate T–62 were round, flat, and opaque and had rough edges. Colonies of the apple leaf isolate were white, glistening, convex, and opalescent; the medium was greened.

POTATO-DEXTROSE-AGAR SLANTS.—On this medium isolates of the blister spot organism, *Phytomonas syringae*, and the magnolia isolate showed moderate growth, which was filiform, glistening, and butyrous and had no odor. The lower end of the streak became light brown in 2 to 3 weeks, and in 5 weeks the entire streak had assumed this color. There was no greening of the medium. Target canker isolate T–64 and the apple leaf isolate produced slight to moderate growth, which was filiform, glistening, and butyrous. With target canker isolate T–62, growth was slight, filiform, flat, gray, and viscid. There was no brown discoloration of the medium.

SODIUM TAUROCHOLATE AGAR.—After 24 hours, growth of the blister spot organism, *Phytomonas syringae*, and the magnolia, target canker, and apple leaf spot isolates was only faintly visible on slants of sodium taurocholate agar (pH 6.8) prepared according to the directions of Patel (*23*). After 48 hours, growth of the blister spot organism, *Ph. syringae*, magnolia isolate, and apple leaf spot isolate was moderate, but in 3 days it was abundant. Growth was filiform, glistening, and smooth, with a bluish cast. In sodium taurocholate poured plates, the colonies of the blister spot organism, *Ph. syringae*, magnolia isolate, and apple leaf isolate were round, smooth, entire, convex, and bluish with a deeper blue center. Streaks of the blister spot organism showed heavy rugose growth after 4 days (fig. 4, *C*). Target canker isolate T–62 produced scant growth. The growth of *Staphylococcus citreus* (Migula) Bergey et al., a Gram-positive organism used as a check, was almost completely inhibited in this medium.

BEEF-EXTRACT-AGAR STABS.—In stab cultures of beef-extract agar (pH 6.8) growth of the blister spot organism, *Phytomonas syringae*, and the magnolia, apple leaf, and target canker isolates was slow; at the end of 8 days it was visible for less than one-fourth the length of the needle track. All the isolates produced circular colonies, at first white but later becoming gray, on the surface of the medium at the point of inoculation. There was no greening or liquefaction of the medium in any of the cultures.

BEEF-INFUSION-GLYCERIN AGAR.—On beef-infusion-glycerin agar (pH 6.9) the blister spot organism made rapid growth, spreading over the surfaces of the slant within 48 hours (fig. 4, *D*). The medium assumed a pronounced green fluorescence within 48 hours. *Phytomonas syringae*, the apple leaf isolate, and the target canker isolate T–64 produced moderate growth during a similar period. Growth was filiform, flat, glistening, and butyrous, and there was a slight greening of the medium. Growth of target canker isolate T–62 was filiform, scant, and viscid, and there was no greening of the medium.

ASPARAGIN MEDIUM.—All the isolates were grown in asparagin medium No. 2, as recommended by Georgia and Poe (*14*). Growth was abundant and a greening of the medium was apparent after 48 hours with all the isolates except target canker T–62. The greening of this medium by the apple leaf isolate was very intense.

USCHINSKY'S SOLUTION.—All the isolates clouded this medium after 24 hours, and a pellicle formed after 48 hours. All but target canker T–62 greened the medium after 48 hours and produced abundant white precipitates.

FERMI'S SOLUTION.—All the isolates except target canker T–62 produced moderate growth and greened the medium after 48 hours. A heavy pellicle was produced by the blister spot organism, *Phytomonas syringae*, the magnolia isolate, and target canker isolate T–64.

COHN'S SOLUTION.—This medium supported the growth of only three isolates of the blister spot organism. Where growth was present it was scant and was evidenced by a faint white clouding. Growth of *Phytomonas syringae*, magnolia isolate, and target canker isolate T–64 was evidenced by faint, white clouding. Target canker isolate T–62 did not grow in this medium.

MODIFIED CZAPEK'S MEDIUM.—This medium was prepared according to the formula for Czapek's solution except that tyrosine was used to supply nitrogen and sodium succinate was used to replace the sugar ordinarily added. Fifteen isolates of the blister spot organism, the Illinois and Netherlands strains of *Phytomonas syringae*, and target canker isolates T–62 and T–64 were inoculated into this medium. In 7 days a faint pink discoloration of the medium was evident in the tubes inoculated with the blister spot, *Ph. syringae*, and the apple leaf isolates. In 21 days the media in these tubes were mahogany brown in color. The change in color from pink to mahogany brown was due to enzymic action, in this instance to the formation of tyrosinase. The tubes inoculated with the target canker isolates showed no change in color in 7 days, although growth was moderate, and only a faint pink discoloration in 21 days. The New York strain of *Ph. syringae* and the magnolia isolate were not grown in this medium.

MALACHITE GREEN AGAR.—This medium was prepared by adding to Wilson's basal medium No. 2 (*34*) 10 gm. of dextrose, 14 gm. of agar, and malachite green so as to make a dilution of 1 to 100,000. The pH was adjusted to 6.9. The blister spot organism, *Phytomonas syringae*, and magnolia isolates made good growth, which was flat, butyrous, and opalescent. No pigment was produced. The malachite green color gradually disappeared and at the end of 18 days none remained. Target canker isolates T–62 and T–64 and the apple leaf isolate failed to grow in this medium.

MISCELLANEOUS BIOCHEMICAL REACTIONS

GELATIN LIQUEFACTION.—With the blister spot organism, *Phytomonas syringae*, the magnolia, apple leaf spot, and target canker T–64 isolates, liquefaction of plain Bacto gelatin stabs (pH 6.9) began in 24 to 48 hours. The surface of the medium was at first crateriform, later becoming stratiform. The surface layers showed green fluorescence. Liquefaction was four-fifths complete in 2 weeks and became complete in 4 weeks. In freshly isolated cultures of the blister spot organism complete liquefaction occurred within 3 weeks. Target canker isolate T–62 failed to liquefy gelatin.

HYDROLYSIS OF STARCH.—Streak inoculations were made in beef-extract agar containing 0.2 percent soluble starch. In one series, which included all the organisms, after 5 days' growth the surfaces of the plates were flooded with a saturated solution of iodine in 50 percent alcohol. A second series was similarly treated after 10 days. None showed any clear zones outside the area of growth, indicating that no diastatic action had occurred.

NITRATE REDUCTION.—Tests of the various isolates were made on 1-, 2- 5-, 7-, 10-, and 14-day-old cultures. The α-naphthylamine sulfanilic acid test was employed. There was no reduction of nitrate by any of the isolates. When *Escherichia coli* (Migula) Castellani and Chalmers was used as a check organism, nitrites were produced from nitrates.

INDOLE PRODUCTION.—The isolates were tested for indole production on Bacto-tryptophane broth (pH 6.8) by the Ehrlich-Bohme test. Tests made at the end of 7, 12, and 18 days were negative. When *Escherichia coli* was used as a check organism, a positive test for indole was obtained.

AMMONIA PRODUCTION.—Strips of filter paper saturated with a freshly prepared Nessler's solution and hung over 24-, 36-, and 48-hour beef-extract-broth cultures of the different isolates gave a strong ammonia reaction in case of all except target canker T–62.

HYDROGEN SULFIDE PRODUCTION.—Strips of lead acetate paper failed to blacken when hung over beef-extract-broth cultures of the different isolates, indicating that hydrogen sulfide was not being produced. Similar tests were made of the different isolates grown on lead acetate agar slants, but the results were negative. *Escherichia coli* used as the check organism gave a positive test for hydrogen sulfide.

REACTION IN MILK.—Fresh skimmed milk sterilized on 3 successive days for 20 minutes at 100° C. was used in these tests. No acid was produced by any of the isolates. Curd formation was evident in the *Phytomonas syringae* and magnolia isolates. An alkaline reaction was observed in 48 hours. *Ph. syringae* and the magnolia isolate cleared the milk in bands, but the blister spot and the other isolates did not.

REDUCTION OF LITMUS.—Litmus milk was blued and a soft coagulum was formed within 6 days by all the isolates except target canker T–62. Within 2 weeks peptonization had begun. *Phytomonas syringae* and the magnolia isolate progressed downward in bands. Reduction of litmus was complete in 4 weeks with all the isolates except target canker T–62, which failed to reduce it. A dark-blue color was evident in 5 weeks in the remaining cultures, and this color was still present when the cultures were discarded 2 months later. While litmus was reduced and a deep-blue color was at first apparent in the tubes inoculated with the target canker isolate T–64, this color changed to blue green after 2 months.

REDUCTION OF BROMCRESOL PURPLE.—Bromcresol purple indicator, at the rate of 1 cc. of a 1.6 percent alcoholic solution per liter, was added to fresh skimmed milk. The medium was adjusted to pH 7.0 with bromthymol blue and then sterilized at 15 pounds' pressure for 15 minutes. Six days after inoculation with the different isolates there was no indication of any acid production. Within 2 weeks the cultures had assumed a wine-red color with reduction complete. No acid reaction was evident.

TOLERATION OF ACID AND ALKALI.—Tests were made in beef-extract broth with a series of hydrogen-ion concentrations from pH 5.0 to 9.4. Growth of the blister spot, *Phytomonas syringae*, magnolia, apple leaf, and target canker isolates occurred in this medium from pH 5.0 to 9.4, with the optimum growth at pH 7.0.

RELATION TO FREE OXYGEN.—The isolates were grown in Smith fermentation tubes containing 1 percent dextrose broth. Growth took place first in the open arms, later progressing toward the domes. These results indicate that none was strictly aerobic. No gas was produced by any of the isolates.

The results summarized in table 4 show that all the organisms with the exception of target canker T–62 exhibited similar biochemical reactions. It would appear therefore that the miscellaneous media under discussion were of little value in distinguishing species. The data, however, seem to indicate a close similarity between the blister spot, *Phytomonas syringae*, the magnolia, and the apple leaf isolates. The apple leaf isolate and target canker isolate T–64 also closely resemble each other. Motility of the latter organism was not demonstrated (table 1). Target canker isolate T–62 because of its non-motility and its failure to liquefy gelatin and to produce a greening of beef-infusion medium is obviously unlike any of the others and must be considered an unrelated organism.

TABLE 4.—*Summary of miscellaneous biochemical reactions [1] of the blister spot and other organisms*

| Organism | Isolate No. | Gelatin liquefaction | Milk | | | | Hydrogen sulfide production | Ammonia production | Nitrate reduction | Indole production | Facultatively anaerobic |
			Curd formation	Peptonization	Acid	Alkaline					
Blister spot	[2] 14	+	−	+	−	+	−	+	−	−	+
Phytomonas syringae	SI–1	+	+	+	−	+	−	+	−	−	+
Do	SH–2	+	+	+	−	+	−	+	−	−	+
Do	NY	+	+	+	−	+	−	+	−	−	+
Target canker	T–62	−	−	+	−	+	−	+	−	−	+
Do	T–64	+	−	+	−	+	−	+	−	−	+
Magnolia	MG–19	+	+	+	−	+	−	+	−	−	+
Apple leaf	A–20	+	−	+	−	+	−	+	−	−	+

[1] + indicates reaction; − no reaction.
[2] 17 other isolates of the blister spot organism gave similar reactions.

REACTION TO DRYING, SUNLIGHT, AND TEMPERATURE

RESISTANCE TO DESICCATION.—Loopfuls of an aqueous suspension of the blister spot, *Phytomonas syringae*, magnolia, apple leaf, and target canker isolates were placed on each of 12 sterile cover slips.　These were placed in a sterile Petri dish, which was then put in a container from which light was excluded.　After 24 hours 4 of the cover slips were taken out and 2 of them were dropped into nutrient broth and the other 2 into melted beef-infusion agar.　There was no clouding of the broth, nor was there any growth in the poured beef-infusion plates.　This procedure was repeated 3 times with identical results.　After 48 hours the procedure was again repeated.　There were no clouding of the broth and no growth in the beef-infusion agar.　From the results obtained under these conditions it was apparent that these isolates were not viable after a 24-hour desiccation.

EFFECT OF SUNLIGHT.—Heavily seeded beef-infusion-agar plates of the different isolates were placed on crushed ice and exposed to direct sunlight for periods of 5, 10, 15, 25, 30, and 40 minutes.　Black paper was placed over half of each glass lid during the exposure.　The toxic action of the sun rays was not apparent on the plates of any of the isolates exposed for 5 or 10 minutes.　There was, however, a gradual diminution in the number of colonies developing in the plates receiving 15-, 20-, and 25-minute exposures.　In the plates exposed for 30 minutes no colonies of the blister spot, magnolia, apple leaf, or target canker T-62 and only a few of *Phytomonas syringae* and target canker T-64 isolates developed.　No colonies of any of the isolates tested ever developed on the exposed portion of the plates subjected to direct sunlight for 40 minutes.　All the organisms made good growth in the portions of the plates covered with black paper.

TEMPERATURE RELATIONS.—The effect of temperatures on the growth of the various isolates was observed by placing the bacteria on the surface of the beef-infusion agar by means of a platinum loop, incubating the plates at the desired temperatures, and measuring the diameter of the colonies at intervals.　The increase in diameter of the colonies of all the isolates was greatest at 27° C.　Within 24 hours clouding was visible in beef-infusion broth cultures from 9° to 30°.　The minimum temperature for growth in beef-infusion-broth was 3.5° and the maximum was 34.5°.　The thermal death point was 52° for the blister spot, *Phytomonas syringae*, magnolia, and apple leaf isolates.　The thermal death points of the target canker isolates were not determined.

CARBOHYDRATE FERMENTATION

In the carbohydrate fermentation studies the peptone-free medium described by Ayres, Rupp, and Johnson (*2*) but slightly modified as given by the Society of American Bacteriologists (*32*) was used. Thirty-one carbon sources, as shown in table 5, were used as 1-percent additions to the synthetic basic liquid medium. Before and after adding the carbon source the hydrogen-ion concentration was adjusted to approximately neutral by adding sodium hydroxide. Bromcresol purple was then added to the media as an indicator. Duplicate cultures were made in all cases.

TABLE 5.—*Carbohydrate reactions* [1] *of blister spot and various other isolates*

Organism	Isolate No.	Monosaccharides							Disaccharides				Trisaccharides		Polysaccharides				Alcohols				Glucosides			Organic acids						
		Arabinose	Rhamnose	Xylose	Dextrose	Levulose	Galactose	Mannose	Sucrose	Maltose	Lactose	Trehalose	Raffinose	Melezitose	Starch	Inulin	Dextrin	Glycogen	Glycerol	Mannitol	Dulcitol	Sorbitol	Arbutin	Salicin	Esculin	Citric	Malic	Lactic	Formic	Succinic	Tartaric	Acetic
Blister spot [2]	14	+	o	+	+	+	+	+	+	gr	o	gr	gr	gr	o	gr	gr	gr	+	+	gr	+	o	gr	gr	–	–	–	–	–	o	–
Phytomonas syringae [4]	NY	+	o	+	+	+	+	+	+	gr	o	gr	gr	gr	o	gr	gr	gr	+	+	gr	+	o	gr	gr	–	–	–	o	–	o	o
Target canker	T-62	o	o	+	+	+	+	+	o	gr	o	gr	o	gr	o	gr	gr	o	+	+	gr	o	o	o	o	–	–	o	o	o	o	–
Do	T-64	+	o	+	+	+	+	+	+	gr	o	gr	o	gr	o	gr	gr	gr	+	+	gr	+	o	o	o	–	–	–	–	–	o	–
Magnolia	MG-19	+	o	+	+	+	+	+	+	gr	o	gr	gr	gr	o	gr	gr	gr	+	+	gr	+	o	gr	gr	–	–	–	o	–	o	o
Apple leaf	A-20	+	o	+	+	+	+	+	+	gr	o	gr	gr	gr	o	gr	gr	gr (³)	+	+	gr	+	o	+	+	–	–	–	–	–	o	–

1 +=Acid reaction; —=alkaline reaction; 0=no growth; gr=growth but no change in reaction.
2 18 isolates gave similar results.
3 Blister spot isolates showed alkaline reaction after 21 days.
4 3 isolates gave similar results.

To prevent their breaking down during sterilization, the sugars lactose, levulose, maltose, sucrose, and raffinose were sterilized by filtration. The basic medium was sterilized by heat and a sufficient quantity of filtered solution of the sugar was then added to each tube. The prepared media were held for 1 week at 25° C. to allow contaminates to develop if any were present. The other carbohydrate media were sterilized in the autoclave at 12 pounds' pressure for 15 minutes.

In all the fermentation studies with the sugars, alcohols, and glucosides, change of color from purple to yellow was considered to be the index of fermentation. With these methods such a change could be attributed only to the fermentation of the carbon source added to the basic medium, since no other energy source was present. All the cultures were under observation for 21 days before being discarded.

The media with organic acid sources of carbon were prepared according to Ayres, Rupp, and Johnson's method (*2*), except that the formula was modified by using 0.15 percent acid instead of the amount originally recommended. Before and after the carbon sources were added, the media were adjusted to pH 7.0 by the addition of sodium hydroxide. The criterion for fermentation was the presence of growth as indicated by turbidity and an increase in alkalinity.

The final results of the fermentation tests appear in table 5.

The 31 carbohydrates used in the studies give a very wide range of comparison of the fermentative ability of the different isolates. Among the isolates of the blister spot organism there was some variation in rates of reaction, but not in the ultimate results. The 3 isolates of *Phytomonas syringae* were identical in their reactions.

A comparison of the reactions of the blister spot and *Phytomonas syringae* isolates shows that the blister spot isolates produced an alkaline reaction slowly in glycogen; *Ph. syringae* grew in this medium, but there was no change in reaction. *Ph. syringae* grew in salicin, but again there was no change in reaction; it did not grow in esculin. The blister spot isolates grew and produced an acid reaction in both of these media. They also grew in all of the organic acid media except tartaric acid and produced an alkaline reaction. The *Ph. syringae* isolates failed to grow in formic, tartaric, and acetic acids. With the exception of these few differences, the blister spot and *Ph. syringae* isolates exhibited similar reactions in all the carbohydrate media used.

A comparison of the fermentative reaction of *Phytomonas syringae* and the magnolia isolate shows that they are indistinguishable. As has been shown earlier, these two organisms also exhibit similar morphological, pathological, and cultural characteristics.

Target canker T–64 differed from the blister spot organism by its failure to utilize salicin and esculin. Target canker T–62 differed by its failure to utilize arabinose, sucrose, glycogen, sorbitol, salicin, and malic, lactic, formic, and succinic acids. It is, therefore, obviously unrelated to the target canker isolate T–64 or to any of the other isolates which have been investigated.

The apple leaf isolate differed from the blister spot isolate by its failure to utilize glycogen, salicin, and esculin. It differed from the *Phytomonas syringae* and magnolia isolates in its ability to grow in formic and acetic acids.

DISCUSSION AND CONCLUSIONS

Rose (*28*) concluded from certain pathological and physiological tests that the differences between blister spot and the disease of apple bark which he called scurfy bark, were of degree rather than of kind. He suggested, however, that more work was necessary before the question of relationship could be settled. Rhoads (*25*) stated in 1924 that he could find neither the scurfy bark canker nor the blister spot disease in the orchards at Mountain Grove, Mo., where Rose had carried on his original investigations with the blister spot disease 8 years earlier.

Since, as has been pointed out, the blister spot disease is inconspicuous and may easily be confused with the minute lesions often produced by the apple scab fungus *Venturia inaequalis*, it is the opinion of the writer that the disease may have existed at the Missouri State Fruit Experiment Station, at Mountain Grove, from the time of its discovery in 1916 until it was rediscovered there in 1938.

That phase of the blister problem dealing with the scurfy, or rough, bark disease has been investigated not only by Rose but also by Hopkins [13] in South Africa and by Lacey and Dowson (*20*) in England. Hopkins concluded from his comparative physiological studies that the Elgin organism and the blister spot and scurfy bark organisms, while closely related, were, nevertheless, distinct species. Lacey and Dowson concluded that the bacterial organism which they isolated from cankers on seedling apple trees, designated as the Wisly strain, was identical with Rose's rough bark organism (*Phytomonas papulans*). They assumed that this latter organism was the cause of both blister spot and the scurfy bark disease. To date Rose is the only investigator who has advanced any proof that a relationship exists between blister spot of apple and the bark disease. It appears, in view of the evidence at hand, that the status of the scurfy bark disease is uncertain and that the blister spot disease of apples is the only disease that has been proved definitely to be caused by the organism originally called *Ph. papulans*.

In the writer's studies of two isolates from target cankers, provisionally designated as *Phytomonas papulans* by Roberts (*26*), and in further studies by Wilson (*34*), neither one was pathogenic to any of the apple varieties inoculated. These two isolates differ from each other culturally and physiologically and also from the other isolates studied in this investigation. They are regarded as two distinct species unrelated to the apple blister spot pathogen.

Studies of the magnolia isolate have shown it to be indistinguishable morphologically, culturally, and physiologically from *Phytomonas syringae*. The pathogenicity of isolates from the two hosts is about the same, since they are cross-inoculable on lilac and magnolia. Both are pathogenic to apple fruit and twigs. A review of the literature does not reveal any reference to a bacterial disease of magnolia. Magnolia, therefore, constitutes a hitherto unreported host for *Ph. syringae*.

The isolate from leaf spot on Rome Beauty apple exhibited cultural reactions similar to the blister spot, *Phytomonas syringae*, magnolia, and target canker T–64 isolates, but differed from them and from

[13] See footnote 4.

target canker isolate T–62 in certain physiological reactions. Inoculations of apple fruits and leaves and plum twigs were attempted at various times during the season with this isolate, but none was successful. These cultural differences and its apparent nonpathogenicity are sufficient reasons, in the writer's opinion, to consider it an unrelated organism.

Studies showed that the blister spot organism remained viable on fruits placed outdoors from October 16 to March 15. After that time the fruit tissues had largely disintegrated and reisolations were not successful. It is obvious that the organism would have to survive at least until May 1 to initiate infection on young fruits of the next crop. It is concluded, therefore, that blister spots on overwintered fruits probably do not constitute a source of infection in the spring.

The spread of the blister spot disease from other possible hosts to apple has been considered and is worthy of further study. Such a study should include an investigation of the pathogenicity of the blister spot organism on wild as well as on cultivated hosts such as was made with *Phytomonas syringae* by Van Hall (*15*), by Fawcett, Horne, and Camp (*13*), and by Smith and Fawcett (*31*).

As previously stated, the blister spot pathogen and *Phytomonas syringae* are slightly different in cultural and morphological characters. The two organisms proved to be cross-inoculable on apple and lilac. *Ph. syringae* when inoculated into apple fruits was pathogenic but failed to produce the papillae characteristic of inoculations with the blister spot pathogen. The blister spot pathogen has proved to be pathogenic on lilac foliage and shoots but failed to produce the wilting typical of *Ph. syringae*. Both these organisms were pathogenic when inoculated into apple twigs. From the foregoing studies it is apparent that the blister spot pathogen is very closely related morphologically and culturally to *Ph. syringae*. The differences in pathogenic behavior appear to be mainly in degree rather than in kind.

In attempting a classification of the blister spot pathogen three possibilities have been considered: (1) Retaining specific rank for *Phytomonas papulans*, (2) considering the blister spot pathogen as identical with *Ph. syringae*, and (3) considering the blister spot pathogen as a variety of *Ph. syringae*. It is the writer's opinion that, because of its similarity to *Ph. syringae* morphologically, culturally, physiologically, and pathogenically, *Ph. papulans* should not retain specific rank. On the other hand, it exhibits enough cultural, physiological, and pathogenic differences to justify its separation from *Ph. syringae*. Because of these differences, *Ph. papulans* is considered a variety of *Ph. syringae*. An emended description of the proposed variety follows.

<div align="center">TECHNICAL DESCRIPTION</div>

Phytomonas syringae papulans [14] n. var.

(*Pseudomonas papulans* Rose; *Phytomonas papulans* (Rose) Bergey et al.)

Short rod, 0.8μ to 2.5μ long by 0.8μ wide, usually occurring singly but occasionally in pairs or short chains. Motile by one to several polar flagella; no spores or capsules demonstrated; Gram-negative but stains readily with ordinary bacterial

[14] If Dowson's (*11*) proposed system of classification is accepted the name of this organism would be *Pseudomonas syringae* var. *papulans*.

stains; not acidfast; forms round, white, opalescent colonies on beef-infusion agar; clouds bouillon and forms a pellicle; liquefies gelatin; produces green fluorescence in beef-infusion and asparagin media and glycerin agar; produces mahogany-brown color in modified Czapek media; reduces litmus and bromcresol purple milk; produces ammonia but no indole or hydrogen sulfide; no nitrate reduction; no diastatic action on starch; grows in Uschinsky's and Fermi's solutions but very feebly or not at all in Cohn's solution; produces acid from arabinose, xylose, dextrose, levulose, galactose, mannose, sucrose, glycerol, mannitol, sorbitol, salicin, esculin, but not from rhamnose, maltose, lactose, trehalose, raffinose, melezitose, starch, inulin, dextrin, dulcitol, or arbutin; produces alkaline reaction in glycogen and in citric, malic, lactic, formic, succinic, and acetic acids; no gas production; facultative anaerobe; optimum, minimum, and maximum pH values for growth 7.0, 5.0, 9.4, respectively; optimum, minimum, and maximum temperatures for growth 27°, 3.5°, 34.5° C., respectively; thermal death point 52°.

SUMMARY

The blister spot disease of apples was first described from Missouri in 1916; it was attributed to a bacterium, *Pseudomonas papulans* (*Phytomonas papulans* (Rose) Bergey et al.). The disease has been reported only in the United States, from Missouri, Arkansas, Indiana, Pennsylvania, Virginia, and Illinois, and has been found to occur under natural conditions only on apple fruits.

The disease is first apparent as a blister spot surrounded by a water-soaked area. The lesion may extend 0.2 to 0.4 mm. below the cuticle. Immediately beneath the diseased area a phellogen layer, from 3 to 5 cells in thickness, develops. The bacteria are present intercellularly throughout the region of the papules. In later stages the epidermis over the blister spot becomes black, dies, and breaks loose from the surrounding healthy tissue. The fact that the blisters are small and shallow seems to indicate that the blister spot pathogen is a relatively weak parasite.

The pathogenicity of the blister spot organism to wounded and nonwounded immature apple fruits was demonstrated by field inoculations. The incubation period for fruit inoculations averaged 21 days. Wound inoculations of immature fruits of apple, plum, cherry, and of tomato fruits in moist chambers were successful. Wound inoculations of twigs of apple, cherry, pear, plum, and lilac and of leaves of apple, peach, magnolia, and lilac were also successful.

Needle-puncture inoculations of fruits and twigs of apple with the lilac blight organism (*Phytomonas syringae*) were successful. An isolate from an undescribed leaf spot disease of magnolia proved pathogenic to apple fruit and twigs and to lilac and magnolia foliage. Two isolates from target canker of apples did not prove pathogenic to fruit and twigs of apple. An isolate from an undescribed leaf spot on Rome Beauty apple foliage failed to infect apple foliage, twigs, and fruit.

Studies showed that the blister spot organism remained viable on fruits placed outdoors from October 16 to March 15. After that time the fruit tissues had largely disintegrated and reisolations were not successful. It is concluded that blister spots on overwintered fruits probably do not constitute a source of infection in the spring.

A study was made of the morphology and physiology of 18 isolates of the blister spot organism, 3 of *Phytomonas syringae*, 2 from apple target cankers, 1 from an undescribed leaf spot of magnolia, and 1 from an undescribed leaf spot on Rome Beauty apple foliage. The

blister spot, *Ph. syringae,* and the magnolia leaf spot isolates were found to bear close morphological resemblances. The target canker and Rome Beauty apple leaf spot isolates appeared to be unrelated organisms.

A study was made of the cultural characters of the various isolates on beef-infusion agar and broth, on potato-dextrose, Patel's, glycerin, and malachite-green agars, and in Uschinsky's, Fermi's, Cohn's, and asparagin solutions. Biochemical reactions were studied on gelatin and milk media and on those for the production of ammonia, hydrogen sulfide, and indole, for the hydrolysis of starch, and for the reduction of nitrates. In the fermentation studies 31 carbon sources were utilized.

The results of the studies of the physiology of the various isolates indicated that the blister spot, *Phytomonas syringae,* and the magnolia isolates are very closely related. The target canker and the Rome Beauty apple leaf isolates are regarded as unrelated species.

It is concluded that the blister spot organism, because of its morphological, cultural, physiological, and pathogenic similarity to *Phytomonas syringae,* should not retain specific rank. On the other hand, it exhibits enough cultural, physiological, and pathological differences to justify its separation from typical *Ph. syringae.* Because of these differences, the blister spot organism is considered a variety of *Ph. syringae* and is designated *Ph. syringae papulans* n. var. A description is given.

The isolate from the magnolia leaf spot is considered to be *Phytomonas syringae,* and accordingly × *Magnolia soulangeana* is to be regarded as an additional host for this pathogen.

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CHANGES IN SOME MINERAL CONSTITUENTS OF PECAN NUTS AND THEIR SUPPORTING SHOOTS DURING DEVELOPMENT [1]

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INTRODUCTION

The poor filling of pecan nuts has been recognized by various investigators (*2, 3, 4, 8, 11, 12, 14, 15, 16, 17, 19, 22*).[3] Different factors such as fertilizers, soil moisture, soil type, leaf area, and crop size have been suggested as having an influence on the filling of nuts, but in many respects the reports are contradictory.

Finch (*5*), studying pecan filling and maturity, found nut filling, date of maturity, and preharvest germination to be related to the vegetativeness, or greenness, of the tree during September and October. Vegetativeness seemed to be related to the available nitrogen supply. Later (*6*) he determined the nitrogen and phosphorus content of leaves, shucks, and nuts on four dates in the late stages of nut development and found an inverse relation between nitrogen and moisture in the plant and nut filling. There was no indication that poor filling was associated with a deficiency of phosphorus.

In more recent studies Finch and Van Horn (*7*) determined at intervals during the growing season the nitrogen content of pecan leaves from trees in various degrees of vegetativeness. They found a correlation between the nitrogen content of the leaves during the growing season and the quality of the nuts at harvest, but they concluded that nitrogen alone was not the direct controlling factor in nut filling. A closer correlation was found between the starch stored in fruit-bearing shoots preceding nut filling and the quality of the nuts produced. It was recognized that a number of factors other than nitrogen exerted an influence on the starch-storing ability of the tree and that cultural treatments would have to be considered in relation to environmental factors in order to achieve any considerable practical control over the filling of the nuts.

The writers (*10*) found differences in the filling of pecan nuts when fertilizers were applied at different times during the growing season. This indicated that the time of the intake of the minerals exerted an influence on the physiological processes of the tree. Therefore, the changes in the mineral constituents of pecan nuts during the course of their development and in the supporting shoots were studied in the hope of obtaining additional data on the cause of poor filling of nuts. The data obtained are reported herein.

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MATERIALS AND METHODS

Samples were taken from 78 trees of the Moore variety of pecan, grown on Greenville sandy loam at Albany, Ga. Cultivation and green-manure crops were the same for all the trees; 68 of them were divided into 7 blocks and each block was fertilized at a different time during the growing season, while 1 block of 10 trees was not fertilized. A fertilizer containing 6 percent nitrogen, 8 percent phosphoric acid, and 4 percent potash was applied at the rate of 60 pounds per tree during 1935, 1936, and 1937. The nitrogen was reduced to 4 percent and the rate of application to 50 pounds per tree in 1938 and 1939. Dolomitic limestone was used in the fertilizer to make it nonacid-forming throughout the period.

The rainfall for the growing season in which the samples were taken was slightly above normal and well distributed. There was no evidence that the trees needed additional moisture at any time during the growing season. The trees appeared to be functioning normally, and the crop matured would seem to bear this out, as the trees produced an average of 76 pounds of nuts per tree. These nuts were of good quality, and 98 percent of them were of marketable grade. The mean oil content of the kernels was 73.1 percent.

On the four sampling dates the nuts were in the following stages of development:

July 15, 1939.—Nuts one-third to one-half full size. Shuck, shell, and kernel not yet distinguishable.

August 15, 1939.—Nuts about full size; shells hard; seed coat of kernel developed and filled with endosperm, but little or no discernible embryo formation.

September 15, 1939.—Shucks beginning to separate from the shell on the trees, and many did separate upon drying in the laboratory. Shell appeared mature, and kernel was solid.

October 3, 1939.—Shucks completely separated from the shell. Kernels considered mature, since they had no attachment with the shuck or the tree.

The samples were collected from the trees in the blocks according to date of treatment. The blocks consisted of 10 trees except in one instance where there were only 8 trees. At each sampling date, shoots [4] bearing 4 nuts were taken at random from the east and west sides of each tree. The nuts [5] were then sampled separately from the shoots, and each sample was weighed in the field. The samples were dried at 65° C. to constant weight for moisture determination; after drying they were ground in a Wiley mill to pass a 40-mesh sieve. The ground material was placed in large mason jars and mixed by shaking for 15 minutes in a rotary shaking apparatus.

The total content of ash, nitrogen, phosphorus, calcium, magnesium, and potassium in the nuts and shoots was determined. The standard methods (1) were used for nitrogen, phosphorus, and calcium. The method described by Hillebrand and Lundell (9) was used for magnesium, and that of Scott (13) for potassium.

RESULTS OF ANALYSES

The analytical data show that marked changes in the composition of the nuts and shoots occurred between the dates of sampling. It was recognized that more than one season's results would be required to interpret adequately the variations due to dates of treatment.

[4] The term "shoot" as used in this paper includes the leaves borne on the shoot also.
[5] The term "nut" as used in this paper includes the shuck, shell, and kernel.

However, it appeared that the samples from trees differing only in dates of treatment might be considered as valid replicates for determining the significance of the changes occurring between the dates of sampling.   All the factors causing variance could not be separated from the data, but it was possible to determine the true error plus the interaction, if any, between dates of sampling and treatments.   Thus, with these reservations, the data were subjected to the analysis of variance as described by Snedecor (*18*), and the means for the eight determinations for each date of sampling are reported.   The interaction thus calculated was used for the error term, and in all probability it is too high rather than too low.

The data for the mean percentage composition of the dry matter are given in table 1.   These data converted to grams of constituents per shoot and per four nuts [6] are given in table 2.   In many ways the actual amounts of mineral constituents show the changes involved in the process of the development of the nuts more clearly than does the percentage composition.

TABLE 1.—*Mean composition of pecan nuts during development and of their supporting shoots (dry-weight basis), 1939*

Part sampled and date of sampling	Dry matter	Ash	Nitrogen	Calcium (CaO)	Magnesium (MgO)	Potassium (K₂O)	Phosphorus (P₂O₅)
	$Percent$	$Percent$	$Percent$	$Percent$	$Percent$	$Percent$	$Percent$
Nuts: [1]							
July 15	25.62	7.59	1.295	2.146	0.538	1.654	0.470
August 15	26.12	5.59	.988	1.156	.325	1.271	.370
September 15	32.09	4.98	1.042	.731	.225	1.309	.390
October 3	36.55	4.99	1.117	.690	.226	1.318	.409
Least significant difference (P=0.05)	.85	.17	.050	.042	.019	.154	.015
Least significant difference (P=0.01)	1.16	.23	.068	.057	.025	.209	.021
Shoots: [2]							
July 15	43.37	8.49	2.013	3.088	.544	1.004	.310
August 15	43.62	8.75	2.029	3.385	.518	1.117	.300
September 15	44.55	9.25	1.830	3.589	.521	.996	.328
October 3	43.18	8.99	1.732	3.526	.479	.760	.313
Least significant difference (P=0.05)	([3])	.21	.069	.087	.023	.116	.019
Least significant difference (P=0.01)		.28	.093	.119	.031	.159	.027

[1] The entire fruit including shuck, shell, and kernel.
[2] The terminal growth including 4 leaves.
[3] Not significant according to *F* test.

TABLE 2.—*Mean constituents [1] in pecan nuts during their development and in the supporting shoots, 1939*

Part sampled and date of sampling	Dry matter	Ash	Nitrogen	Calcium (CaO)	Magnesium (MgO)	Potassium (K₂O)	Phosphorus (P₂O₅)
	$Grams$	$Grams$	$Gram$	$Gram$	$Gram$	$Gram$	$Gram$
Nuts: [2]							
July 15	4.33	0.3283	0.0560	0.0928	0.0233	0.0716	0.0204
August 15	13.85	.7745	.1367	.1601	.0450	.1759	.0512
September 15	22.76	1.1332	.2371	.1662	.0512	.2977	.0888
October 3	25.39	1.2664	.2833	.1749	.0574	.3344	.1038
Least significant difference (P=0.05)	.62	.0499	.0127	.0087	.0008	.0237	.0053
Least significant difference (P=0.01)	.85	.0679	.0173	.0119	.0011	.0323	.0073
Shoots: [3]							
July 15	7.75	.6581	.1560	.2394	.0421	.0779	.0240
August 15	8.21	.7185	.1665	.2780	.0426	.0919	.0246
September 15	7.38	.6811	.1349	.2643	.0385	.0734	.0242
October 3	6.69	.6009	.1159	.2359	.0321	.0507	.0209
Least significant difference (P=0.05)	.44	.0397	.0062	.0164	.0031	.0106	.0015
Least significant difference (P=0.01)	.59	.0541	.0085	.0224	.0042	.0144	.0021

[1] The calculated amounts of constituents for 4 nuts and for their supporting shoot.
[2] 4 entire fruits including shuck, shell, and kernel.
[3] The terminal growth including 4 leaves.

[6] Units of 4 nuts were used rather than 1, since all the shoots supported 4 nuts.

In order to evaluate properly the importance of the changes shown by the data, the stage of development of the nuts must be kept in mind. The first period, July 15 to August 15, was one of very active growth during which the nuts made from one-half to two-thirds of their total increase in size. Differentiation of the shuck, shell, and kernel took place, the shells became hard, and the seed coat of the kernel developed and was full of endosperm, but little or no discernible embryo formation occurred. This period was characterized by no significant change in the percentage of dry matter in the nuts, whereas the actual dry weight per nut increased at a highly significant rate. A highly significant decrease in the percentage of nitrogen, calcium, magnesium, potassium, and phosphorus in the dry matter occurred, while the actual amount of these elements per unit of nut increased in highly significant quantities, these results showing that movement of the elements into the nuts was taking place, but at a slower rate than the accumulation of actual dry weight in the nuts.

During this first period the percentage of dry matter did not change significantly in the shoots, whereas there was a significant increase in the dry weight per shoot. There was no significant change in the percentage of nitrogen, potassium, or phosphorus, a significant increase in the percentage of ash, a highly significant increase in the percentage of calcium, and a significant decrease in the percentage of magnesium in the dry matter. Because of a significant increase in the dry weight per shoot, there was a significant increase in the amount of potassium per shoot and a highly significant increase in ash, nitrogen, and calcium while the magnesium and phosphorus did not change significantly.

There was no evidence of a withdrawal of any of these constituents from the shoots by the nuts, and the ash, nitrogen, calcium, and potassium accumulated in the shoots at a more rapid rate than they were being used by the nuts.

During the second period, August 15 to September 15, the nuts increased very little in size, the shuck reached the point of partial separation from the shell, the shell appeared mature, and the kernal became a solid mass of fleshy material.

This period was characterized by highly significant increases in the percentage of dry matter and in the dry weight per unit of nut. These increases were accompanied by a significant increase in the percentage of nitrogen and phosphorus, no significant change in the percentage of potassium, but a highly significant decrease in the percentage of ash, calcium, and magnesium. In actual amount of these constituents per unit of nut, calcium was the only constituent that did not accumulate in the nuts in highly significant quantities. The very rapid intake of nitrogen, phosphorus, magnesium, and potassium into the nuts during this period is of particular interest when considered in the light of the studies of Thor and Smith (20, 21), who found that during kernel development such organic constituents as oil, protein, and acid-hydrolyzable polysaccharides accumulated in the nuts at a very rapid rate. The results indicate a direct correlation between the movement of these organic constituents and that of nitrogen, phosphorus, magnesium, and potassium into the nuts. The very slow rate of intake of calcium into the nuts during this second period

indicates that this element accumulates in the nut before kernel development.

During this period there was no significant change in the percentage of dry matter in the shoots, but there was a highly significant decrease in the dry weight per shoot. There was a highly significant increase in the percentage of ash, calcium, and phosphorus in the dry matter, a highly significant decrease in nitrogen, a significant decrease in potassium, and no significant change in the magnesium. In units per shoot there was a highly significant decrease in nitrogen and potassium, a significant decrease in magnesium, and no significant change in ash, calcium, and phosphorus. These results indicate that the nuts were withdrawing from the shoots all the constituents except calcium and phosphorus at a more rapid rate than they were being replaced.

During the third period, September 15 to October 3, the nuts were filled with kernels which became mature. On the whole, the changes noted for this period were a continuation of those mentioned for the second period. Nitrogen, phosphorus, magnesium, and potassium continued to move into the nut in highly significant quantities, but at a slightly slower rate than during the previous period. Calcium moved into the nuts at a slightly higher rate during this period than during the preceding one.

As in the nuts, the changes in the constituents of the shoots were largely a continuation of those noted in the preceding period, except that during this period the decrease in phosphorus was highly significant, whereas earlier it was not significant. It is interesting to note that during the period of nut filling the actual amounts of the mineral constituents in the shoots decreased at the same time that they were being accumulated in the nuts.

As stated previously, the data on the nuts as reported in this paper were obtained from the whole nut, including the shuck, shell, and kernel. It would have been impossible to separate the two earliest samples into these different parts because differentiation had not taken place. In the last two samples this separation could have been made, and had this been done no doubt a clearer knowledge of the changes associated with the development of the kernel would have been obtained. At the final harvest, samples of nuts were obtained from the trees according to dates of treatment, and separate analyses were made on a composite sample of the shells and kernels from each treatment. These data are presented in table 3.

The percentage of nitrogen in the kernel was highest, followed in order by potassium, phosphorus, magnesium, and calcium. Near maturity, October 3, the percentage of potassium in the whole nut was highest, followed by nitrogen, calcium, phosphorus, and magnesium. These changes in the order of the elements in the kernel as compared with those in the whole nut show that nitrogen and magnesium are deposited in the kernel to a relatively greater degree than are the other elements. This would indicate that a systematic study of the mineral constituents of the shuck, shell, and kernel from the time of differentiation of the tissues to maturity might reveal some interesting relations.

TABLE 3.—*Mean composition* [1] *(dry-weight basis) of mature pecan kernels and shells, 1939*

Part	Ash	Nitrogen	Calcium (CaO)	Magnesium (MgO)	Potassium (K₂O)	Phosphorus (P₂O₅)	Oil
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Kernel_____	1.67±0.07	1.684±0.082	0.107±0.008	0.248±0.006	0.946±0.057	0.318±0.012	73.1±1.1
Shell_____	2.27± .06	.293± .025	1.032± .043	.068± .007	.480± .027	.061± .009	_____

[1] 8 samples collected at harvest.

DISCUSSION

The data in this report and that of Thor and Smith (*20, 21*) on the changes in organic constituents of the pecan during development bring out some interesting correlations. Nitrogen was determined in both of these studies, and there is a strikingly close similarity in the amount found in the nuts at different stages of development and in the trend of the changes recorded. For example, in the Texas study (*21*) the nitrogen content of the nuts was 1.15 and 0.96 percent on July 14 and August 14, respectively, and in this study it was 1.295 and 0.988 percent on July 15 and August 15, respectively. As these were the only two calendar dates that coincided other direct comparisons cannot be made; but it is evident from these data that the course of change in the nitrogen content was similar in the two studies. So close an agreement between studies conducted under such different environmental conditions is rather surprising.

It was pointed out earlier (p. 302) that the period of rapid intake of nitrogen, phosphorus, magnesium, and potassium into the nuts as shown in this study coincides with the period of rapid accumulation of such organic constitutents as oil, protein, and acid-hydrolyzable polysaccharides in the nuts as shown by the study in Texas. In both studies, this period coincided with the period of rapid kernel development. It is indicated that the four elements named above play an important role in the assimilation and storage of the organic constituents that make up the greater part of the pecan kernel. It is conceivable that a deficiency of any of these elements or a lack of balance between them at the time of kernel development might result in poorly filled nuts.

This study shows that the greater part of the calcium in the nuts accumulates there during the period when the nuts are growing in size and that relatively small amounts move into the nuts during the filling period. This would indicate that calcium is more important in the formation of structural tissues than it is in the formation of the kernels. The content of calcium stored in the kernel supports this theory.

The reduction in weight of the pecan shoots and the decrease of minerals in the shoots during the filling of pecans may have an important bearing on the tendency of the pecan to bear biennially. The pecan matures its nuts late in the season, thus leaving very little time for the tree to build up reserves for the next season. This makes it very important that the trees be supplied with sufficient minerals in the proper balance so that the mineral and organic reserves will

not be too greatly depleted in the shoots by a heavy nut crop. A quick recovery of the organic reserves necessary for strong shoot development in the spring may be associated with and dependent on the quantity and balance of the mineral reserves after the harvest of a heavy crop.

SUMMARY

During the growing season eight blocks of pecan trees were sampled on four different dates representing different stages in the development of pecan nuts. Dry matter, ash, nitrogen, calcium, magnesium, potassium, and phosphorus were determined in the nuts and shoots. Data on the composition are reported for different dates, and the changes as related to the stage of the development of the nuts are discussed.

The percentage composition of the pecan nut and of its supporting shoot is influenced to a high degree by the stage of development of the nut. In the last month of the period of growth in size, the actual dry weight of the nut increased at such a rapid rate that the percentage of mineral constituents in the dry matter showed highly significant decreases even though they continued to accumulate in the nut in highly significant quantity on the basis of units per nut. The shoot increased in dry weight and in the content per shoot of all the minerals except magnesium and phosphorus.

In the second and third periods studied, during which the kernel of the pecan developed and filled, nitrogen, potassium, magnesium, and phosphorus accumulated in the nut at rates exceeding the accumulation of dry matter even though the dry weight of the nut itself increased at a rapid rate. Calcium accumulated in the nut in very small quantities. The shoots lost significant quantities of all the minerals.

Data on the composition of pecan kernels and shells show that calcium is deposited largely in the shell while nitrogen, potassium, phosphorus, and magnesium are deposited to a greater extent in the kernel. Composition of the whole nut does not give a true picture of the changes taking place in the nut during kernel development.

The unit basis of expressing the results of analyses such as are reported here was found to be more satisfactory than expressing the results on a percentage basis.

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A POMOLOGICAL AND CYTOLOGICAL STUDY OF A RUSSETED SPORT OF THE STARK APPLE [1]

By C. P. SWANSON, *research assistant in cytogenetics*, and V. R. GARDNER, *head, Department of Horticulture, and director, Michigan Agricultural Experiment Station*

INTRODUCTION

Many of the present day varieties of horticultural fruits owe their origin to bud sports which can readily be propagated by asexual methods (*9*).[2] The hybrid nature of these fruits, particularly in the pome and citrus groups, precludes a detailed genetical analysis of such somatic segregation, with the result that comparatively little can be stated with certainty regarding their mechanism of origin. The lack of genetical data and the small size of the chromosomes also prohibit the recognition of cytological changes other than the gain or loss of whole chromosomes. Duplication or deletion of chromosome segments would not be detected. Jones (*4, 5, 6*), on the other hand, using marker genes in maize endosperm, has demonstrated conclusively that chromosome aberrations can lead to atypical growths as well as color mosaics, both types of which are frequent in pome and citrus fruits.

A recent cytological survey of some bud sports in apple (*Malus pumila* Mill.) has indicated that changes of sufficient magnitude can be pursued to advantage in elucidating the mechanism of bud-sport origin. A single variable bud sport of the Stark apple provides the source of material for this study.

MATERIAL AND METHODS

Incident to the junior author's study of bud sports in Michigan, four solid-red limb variants were found, one each in orchards near Kibbie, Lacota, Fennville, and South Haven, and one russeted sport in the George B. Meechum orchard near Fennville. The present article deals with this russet mutation. A comparatively large part of the sporting portion of the parent tree had been top-grafted at the time it was brought to the junior author's attention in 1927, so that, while cions could be obtained, observations as to the range of variation in character of fruit on the sporting limb could not be comprehensive.

Cions of the russet sport were obtained in 1927 and designated as Stark strain No. 287. Some were set as top-grafts in trees of bearing age in the Graham Experiment Station orchard near Grand Rapids. Others were set on small nursery seedling stock. Two of these nursery-propagated trees were planted in the orchard in the spring of 1932. The top-grafts bore their first fruits in 1933 and fruited regularly thereafter. The first crop of any considerable size produced by the orchard trees was in 1939.

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For the cytological study, material from the nursery-propagated trees was collected in late February 1941 and forced into bud in the greenhouse. These branches had been labeled the preceding fall prior to harvest, and included those of the russet sport, and single whole branches of what appeared to be partial and complete reversions to the original Stark type. Branches from a normal Stark were included for comparison. Fixation was in 3:1 alcohol-propionic acid; the smears were made by the usual acetocarmine method.

POMOLOGICAL STUDY

CHARACTERISTICS OF THE STARK APPLE

The Stark apple, said to have originated in Ohio, has been in cultivation for three-quarters of a century *(1, v. 1, p. 317)*. Though never attaining a commanding position in any important commercial producing district, it has been rather widely planted and is recognized as a more or less standard late-winter variety in the Northeastern States. Many orchards contain trees of this variety numbering in the hundreds.

Characteristically, its fruits have a greenish-yellow ground color and are striped, splashed, and blushed with a rather dull red. Where shaded, the ground color predominates; where well exposed to the sun, the overlying color predominates. Under favorable growing conditions, the skin is smooth and glossy. Under conditions of relatively high humidity, or when certain spray materials are used, the cuticular layer is more or less broken and roughened and a portion of the surface presents a slightly russeted appearance. This normal russeting, if such it may be called, is only partial and seldom covers more than a quarter or a third of the surface. It is thin and generally irregularly distributed over the surface of the fruit.

Though observation indicates that Stark is less given to producing limb sports than many other apple varieties, a number have been reported. In 1932, Shamel and Pomeroy *(9)* listed seven solid-red or high-color variants in the fruits.

CHARACTERISTICS OF RUSSET STRAIN NO. 287

Fruits of the russet bud sport borne by the top grafts in 1933–37 were rather uniformly of normal size and shape for the Stark variety, but differed from the normal in being fairly completely covered with an even, thin coat of russet. Irregular patches or areas of the normal glossy skin were present in a small percentage of the fruits. In the year 1938, these top-grafts again bore a medium crop of fruit, but only two species were like those borne the 5 preceding years. The remainder were of more or less normal shape for Stark, but in size ranged from only one-third to two-thirds normal and were covered with a thick, heavy, coarse russet. The smallest specimens were the most heavily and coarsely russeted and they were also rather deeply cracked, there being a clear correlation between the heaviness of russeting, the depth of the cracking, and the progressively smaller size of the fruits (figs. 1 and 2). The crops borne by these top-grafts in the 1939–42 period were like those of the 1933–37 period, except that an occasional fruit showed a tendency to be heavily and coarsely russeted and more or less cracked. Detailed russeting records of the 1939 and 1940 crops of these top-grafts are presented in table 1.
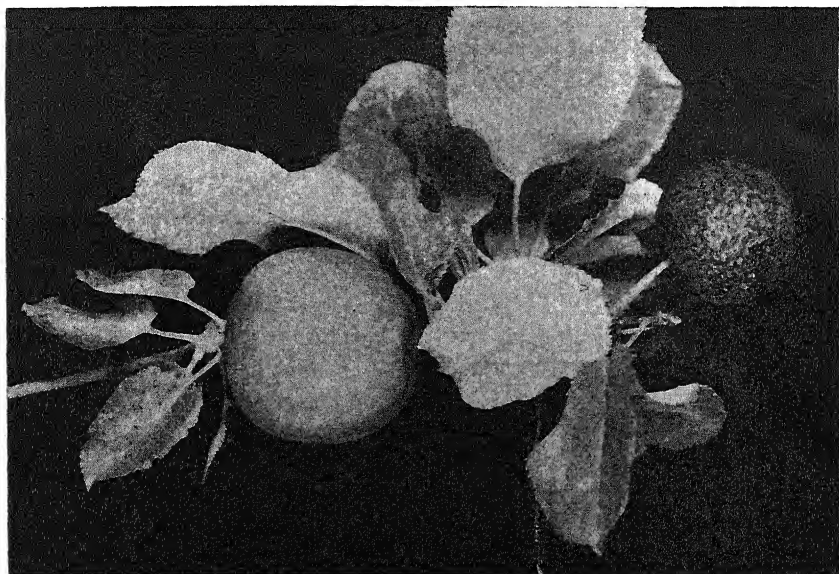
FIGURE 1.—The apple on the left is a thinly and evenly russeted fruit, typical of Stark strain No. 287. The specimen on the right is a typical, small-sized, coarsely russeted and somewhat cracked variant from strain No. 287.
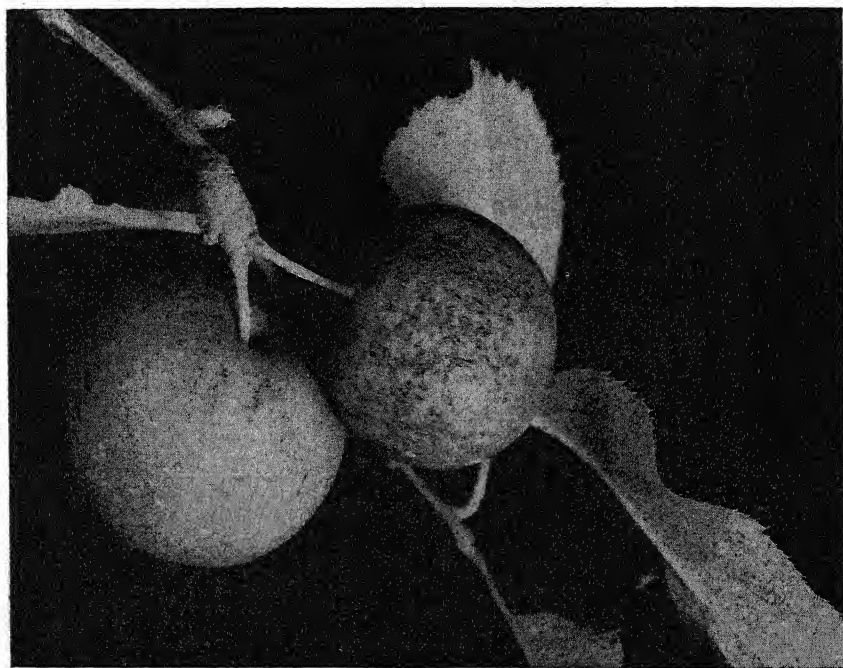
FIGURE 2.—Two fruits borne by a single spur of Stark strain No. 287. The one on the left shows a slight and the one on the right a marked tendency toward coarse russeting and cracking, typical of the entire 1938 crop on the top-grafts of this strain and appearing occasionally in the 1939–42 crops.

TABLE 1.—*Frequency distribution table showing numbers of fruits of Stark strain No. 287, top-grafts and orchard trees, with varying percentages of their surface covered with russet, seasons of 1939–41*

Location in orchard	Year	Percentage of surface covered by russet—							
		1–25		25–50		50–75		75–100	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
Top-graft	1939	8	4	20	10	45	24	118	62
	1940	0	0	9	7	51	43	62	50
Tree B-19	1939	17	12	23	17	28	21	67	50
	1940	0	0	23	12	63	34	99	54
	1941	12	2	3	1	32	6	481	91
Tree B-20	1939	100	31	59	19	59	19	97	31
	1940	0	0	4	4	22	20	83	76
	1941	14	2	8	1	123	--------	487	77

The fruits borne by the 2 orchard trees each year during the 1939–42 period were much like those borne by the top-grafts during the same period. Most specimens were of normal size and shape for the Stark variety and were thinly and evenly russeted. However, distributed irregularly throughout the trees were specimens somewhat cracked (figs. 1 and 2). In 1941 each of the 1,182 fruits borne by the 2 orchard trees was weighed and the amount and density of its russeting estimated, the estimate being largely on the basis of the percentage of its surface that was covered with russet. The correlation coefficient between fruit weight and amount of russeting was 0.364 ± 0.017. The distribution of the heavily russeted fruits was by spur rather than by branch. That is, no whole branches were found that bore the heavily russeted fruits one year, and necessarily produced the same kind of fruits the following year. Occasionally when two fruits were borne by a single spur one would be thinly and evenly russeted, while the other was heavily russeted, as shown in figure 2.

One small branch, springing from a lower horizontal limb of one of the two orchard trees of this strain, was noted in 1939 as producing smooth-skinned, glossy fruits exclusively, thus showing complete reversion to the normal Stark parent. This reverting branch, shown in figure 3, has continued to bear smooth fruits.

CYTOLOGICAL STUDY

The Stark apple possesses a diploid number (fig. 4, *A*, *B*, and *C*), a chromosome number different from that of the usual apple varieties which show multiples of a haploid number 17. This agrees with previous findings of Newcomer (7). Roscoe (8), however, has reported a diploid number of 51, making this variety a triploid. In view of the fact that a diploid number of 42 is aberrant for members of the Pomoideae, the preparations were carefully scrutinized and several additional strains of Stark were examined, but in all instances the same chromosome count of 42 was found. This suggests that possibly 2 cytologically different strains of the Stark apple exist, but since all Stark trees undoubtedly stem from the same origin, this seems unlikely.

Univalents were frequent at metaphase I, but they were usually incorporated into one or the other of the nuclei before telophase reorganization (fig. 4, *A*). Chromatin bridges at anaphase indicated the presence of inversions. Diplotene (fig. 4, *C*) was clear but difficult

to analyze for multiple associations. Trivalents were present at metaphase, with six the maximum recorded.

The russeted sport possessed a complement of 51 chromosomes (figs. 5, *A* and *B*, and 4, *D*, *E*, and *F*). Meiotic configurations were very similar to those described by Roscoe (*8*), with trivalents and quadrivalents frequent. Multiple associations were observable at diplotene (fig. 4, *D*), but at diakinesis (fig. 4, *E*) the looseness of pairing made this uncertain. Interesting to note was the stretched condition of the nucleolus at diplotene and diakinesis (fig. 4, *D* and *E*), a condition also noted before pressure was applied to the cover glass in making the smears. It would appear that a repulsion of bivalents attached
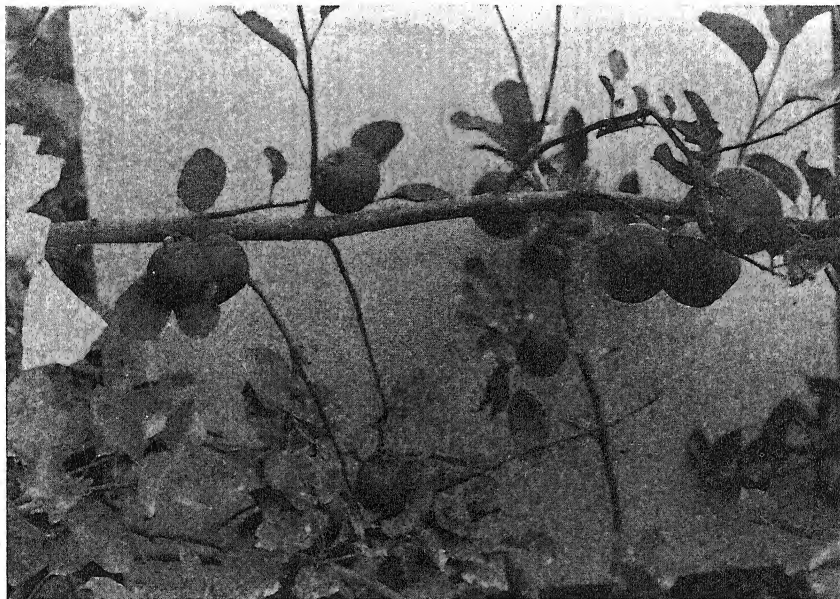
FIGURE 3.—Branch on one of the trees of Stark strain No. 287 that shows a complete reversion to the normal Stark type with smooth, glossy skin. The spur on the left is bearing one typically russeted fruit and one that has varied in the direction of rough, coarse russeting. The point where reversion has occurred is just to the right of the spur on the left that is bearing two fruits.

by their nucleolar constrictions leads to a stretching of the nucleolus from a sphere to a spindle-shaped body. A comparison of cells in comparable stages of prophase shows that the nucleolus is much larger in the russet sport than in the normal Stark strain.

The single branch showing a complete reversion to the parental type (fig. 3) gave a cytological picture similar in every detail to that of its progenitor. Again the chromosome number was 42.

Several branches showing what was assumed to be a partial reversion to the parental type were selected for study. With the exception of one, which was more decidedly in the direction of the original Stark in size, shape, and degree of russeting, all possessed 51 chromosomes. The exceptional branch showed a somatic complement of 46 chromosomes in the cells of the tapetum. Counts of 45 and 47 were made but the majority of them revealed 46 (fig. 5, *C*, *D*, and *E*),

particularly when anaphase figures were utilized. Occasional cells from those flower buds toward the base of the branch had 51 chromosomes, indicating that the basal portion was the place of origin of this mutant branch and that a mixture of cells was present. There was no change in the external morphology of the stem and leaves which pointed to an altered cytological condition, although it is quite possible that the polyploid condition of the apple permits a cytological change without visible morphological expression.
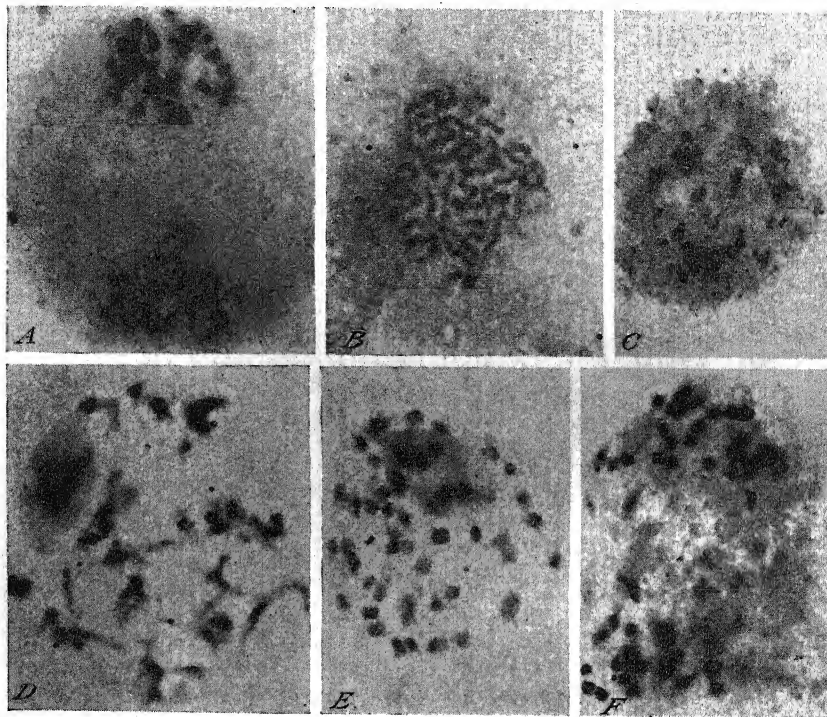
FIGURE 4.—Normal Stark: Anaphase I (*A*), somatic metaphase from tapetal tissue (*B*), diplotene (*C*). Russet bud sport: Diplotene (*D*), diakinesis (*E*), anaphase I (*F*). × 1,000.

DISCUSSION

In a number of respects Stark strain No. 287 is like many bud sports of the apple that have been under study at the Michigan station, e. g., (1) it has remained sufficiently true to type to be classified as a strain or variety distinct from the parent, and (2) it has yielded one or more complete reversions to the parent type. In one respect it has deviated from the usual bud mutant pattern, viz., it has in turn yielded "sports," still more extreme, in the same direction in which it deviated from the parent form. In this latter instance the sporting apparently has taken place in the flower bud primordia, rather than in the leaf buds. This is evidenced by the irregular occurrence and distribution of the coarsely russeted variants and also by their "wholesale" occurrence on the top-grafts in 1938, and in 1938 only, of the 10 years that fruits have been borne by the top-grafts and

trees under study. Presumably the wholesale sporting of that year was due indirectly to some factor or combination of factors of the environment of that season (1938) or of the preceding season while flower bud primorida were being differentiated or developed. That it cannot be attributed to any direct influence of 1938 conditions on russeting per se is indicated by the fact that the mean percentage of russeting (i. e., percentage of surface of fruits covered by russet) on 5,226 fruits of a large 50-year-old Golden Russet tree growing within 200
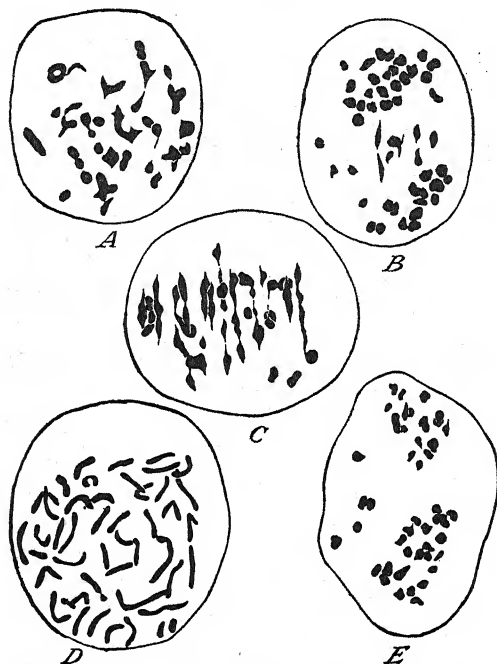
FIGURE 5.—Russet bud sport: Metaphase I (*A*), anaphase I (*B*). Forty-six-chromosome reversion: Metaphase I (*C*), somatic metaphase from tapetal tissue (*D*), anaphase I (*E*). × 1,350.

yards of the top-grafts of strain No. 287 was 9 in 1938, and for its 16,590 fruits in 1939 it was 18. Climatic conditions in 1939 were therefore twice as favorable for russeting as in 1938, when the russeting of the fruits on the Stark strain No. 287 top-grafts was so greatly accentuated.

In view of Jones'(*4, 5, 6*) clarification of atypical growths and color mosaics in maize endosperm, there is sufficient reason for assuming that chromosomal aberrations lead to many of the bud sports arising constantly in horticultural plants. Huskins' (*3*) summary, which indicates that most of the dominant and some of the recessive mutations, so-called, found in polyploids are attributable to chromosomal aberrations affecting appreciable regions of the chromosome, or even whole chromosomes, lends additional support to the belief that bud sports in apples are the result of chromosomal changes since the apple is a derived polyploid, and mutations or bud sports arising somatically must be dominant to be recognized. The present lack

of evidence can no doubt be traced to the inadequacy of the apple as a subject for cytological studies.

The cytological study reported here suggests that the russet sport No. 287 arose from the normal Stark by an addition of nine chromosomes, and that this increase brought with it an unstable genetic condition. The mechanism for this chromosomal increase is not understood, but the instability of the genotype is expressed by the varying degrees of russeting both between seasons and within and between spurs and branches, and also by the varying chromosomal constitution of different parts of a single tree. The russeting variability is strikingly similar to the color, form, and maturity variability found by Gardner (2) in the hetero-chimeric apple sport "Graham," though in the latter the variations when once produced, appear to be more stable.

The 51-chromosome russet sport is therefore a triploid, but not a balanced triploid of the sort described by Roscoe (8). The 42-chromosome normal Stark, on the other hand, is an aneuploid in that its chromosome complement is not a multiple of the haploid number 17. Assuming, however, as a possibility, that the russet bud sport arose as a dominant mutation from a 51-chromosome Stark such as described by Roscoe, it then becomes necessary to account for the origin of the 42- and 46-chromosome branches as well as the fact that the 42-chromosome branch represents a complete reversion to the normal Stark in shape, size, and color. It appears likely, although still difficult to visualize in the light of our present cytogenetical knowledge, that the 51-chromosome form arose from the 42-chromosome normal Stark, possibly through somatic nondisjunction, and that the 42-chromosome reversion is not a reversion at all but merely the result of carrying some of the original parental cells over from the source tree (the normal Stark) at the time of grafting. The inclusion of 51-chromosome cells among those showing 46 at the base of the partial reversion branch suggests further that a loss of 5 chromosomes occurred in the apical meristem just prior to, or at the time of, branch initiation. The fact that this partial reversion branch appeared no different from others on the same tree seemed to indicate that the chromosome loss affected the growth characteristics but little.

It is evident in any event that the russet Stark No. 287 is a complex chromosomal chimera, and that by the outcropping of various tissues having different chromosome numbers, the establishment of variant branches has been brought about. The origin of the variant tissues probably lies in an unstable somatogenesis, conditioned perhaps by a gene unbalance.

SUMMARY

A russeted bud sport of the Stark apple is described. The russeting was generally thin and even, but showed considerable variability within trees and between seasons. Coarse russeting was correlated with deep cracking and a decreased weight of the fruit.

The bud sport showed a tendency to revert to normal as well as to "mutate" toward a more extreme form of russeting. With the exception of one "normal" branch, the other "mutations" appeared to be conditioned by environmental factors.

The bud sport revealed a diploid chromosome number of 51, while the normal Stark showed a diploid number of 42. The branch show-

ing complete reversion to normal possessed a chromosome number of 42, and one branch showing partial reversion had a chromosome number of 46. Other "partially reverted" branches showed no alterations in chromosome number.

It was suggested that the 51-chromosome sport arose from the 42-chromosome Stark (which is an aneuploid) by an addition of chromosomes, possibly through somatic nondisjunction, and that the 46-chromosome branch resulted from a loss of chromosomes in the branch meristem. The 42-chromosome completely reverted branch may have resulted from active cells carried over from the parental tree through the graft.

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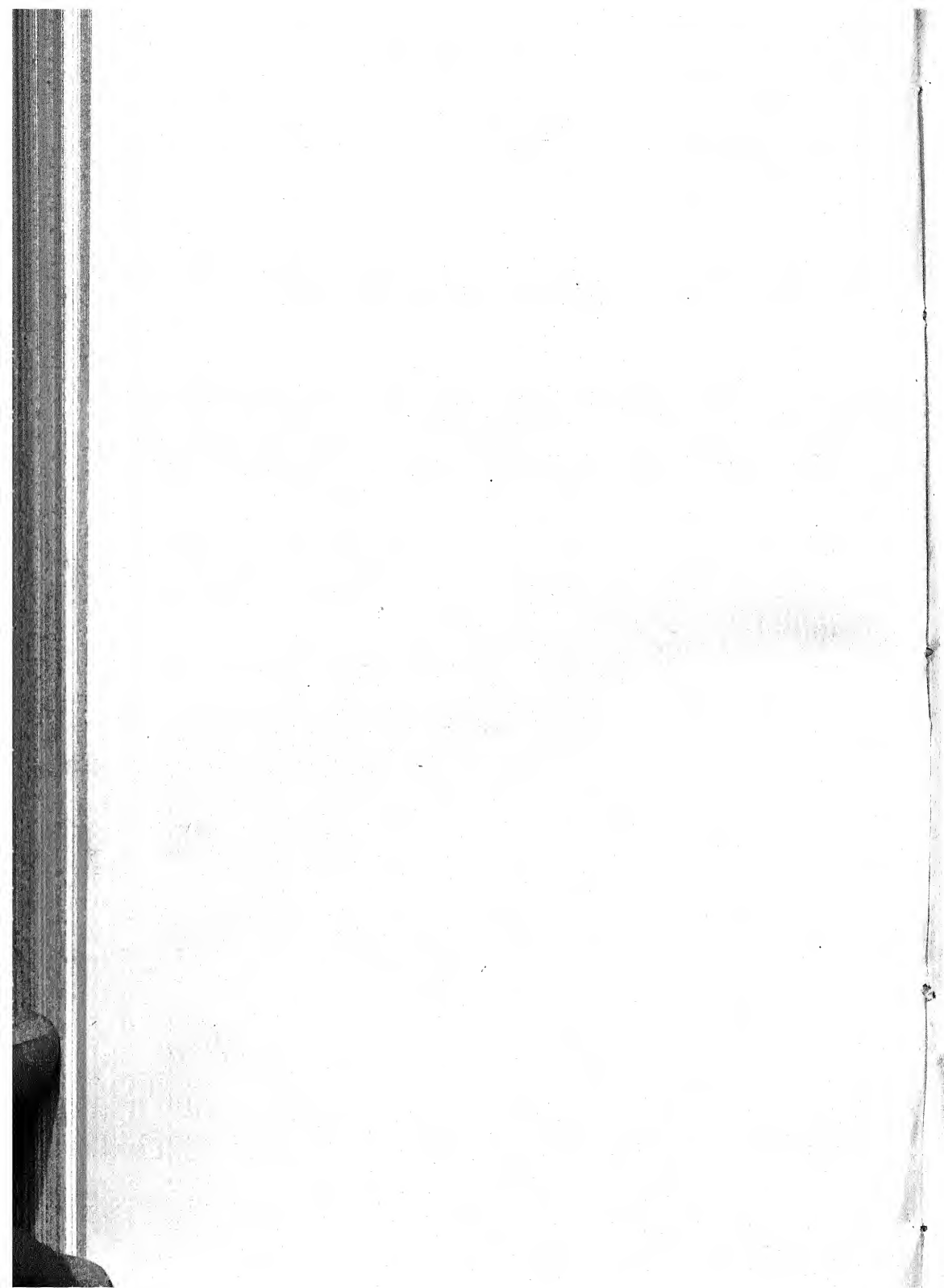
SURVIVAL ON GRASS PLOTS OF EGGS AND LARVAE OF THE STOMACH WORM, HAEMONCHUS CONTORTUS [1]

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INTRODUCTION

The large stomach worm (*Haemonchus contortus*), a destructive parasite of sheep and other ruminants, is acquired by a susceptible animal through ingestion of infective larvae with the feed. Control of this parasite depends on removing the adult worms by suitable anthelmintics or preventing or reducing infection of susceptible animals by improved pasture management or a combination of the two methods. The type of pasture management to be used depends on the extent of survival of infective larvae under natural conditions.

REVIEW OF LITERATURE

Since Ransom (*10*) [2] worked out the life history of *Haemonchus contortus*, a number of studies have been conducted to determine how long eggs or larvae of this species would survive on pasture. Ransom found that larvae kept out of doors for 3 months during the winter showed little reduction in the number of contained food granules. During his experiments, the larvae were frozen for about one-third of the time. Ransom (*11*) also found that "pastures on which infested sheep have grazed will not become free from infestation with the twisted wire worms after remaining empty from October 25 to June 16, climatic conditions being similar to those of Washington, D. C." This writer (*12*) also reported that pastures kept free of ruminants for a year are practically free of stomach worm infection. He considered that infective larvae of *H. contortus* were slightly affected by dryness and freezing. Veglia (*16*), on the other hand, indicated that infective larvae were unable to withstand natural climatic conditions in South Africa, and unless protected by shade or subjected to rainfall many, and often all larvae, were dead after an exposure of less than 1 day. Mönnig (*9*) showed that infective larvae of *H. contortus* died in less than 3 months during dry weather in South Africa.

Dikmans and Andrews (*7*) allowed lambs in the spring to graze on pasture that had been unoccupied during the winter. The lambs became infected with *H. contortus* as well as with some other nematodes. Boughton and Hardy (*3, 4, 5, 6*) stated that in Texas infective larvae of *H. contortus* survived on pastures for 22 months, but after 31 months the lambs allowed to graze on a pasture previously infested remained free from infection. Griffiths (*8*), using uninfected lambs for testing the survival of larvae on paddocks, found that *H. contortus* larvae did not survive from October to May on Montreal

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[1] Received for publication February 8, 1943.
[2] Numbers in parentheses refer to Literature Cited, p. 323.

Island, Canada. Swales (*14*) confirmed Griffiths' work and found that in eastern Canada larvae of *H. contortus* apparently did not survive over a period lasting from fall (August to November) until the following June. Baker (*1, 2*) stated that at Ithaca, N. Y., worm-free lambs became infected with *H. contortus* and other worms when allowed to graze on a pasture 12 months after heavily infested sheep had grazed on it until all grass was eaten down to the crowns. This author succeeded in infecting two lambs by allowing them to graze on this pasture 21 months later. Taylor (*15*), in reporting experiments carried out in 28-inch-square boxes subdivided into 4-inch-square grass plots and inoculated September 6, 1937, with "ovine trichostrongylid larvae," stated that death of the larvae was constant and rapid. Viable larvae were found as late as 133 days after the boxes were prepared. The identity of the larvae was not given.

The following is a summary of the survival periods, as determined by the various investigators, of the preparasitic stages of *H. contortus*: 22 months but not 31 months by Boughton and Hardy; at least 21 months by Baker; at least 8 months by Ransom—he believed that pastures would be practically free of the infective larvae after 1 year; over the winter by Dikmans and Andrews; less than over the winter by Griffiths and by Swales. Boughton and Hardy found a total of only three adult *H. contortus* in two lambs that had grazed on a pasture contaminated 22 months previously by sheep infected with the nematode in question. The number of *H. contortus* surviving in Baker's experiments is not known as the lambs used in the test were not removed from the pasture before the worms acquired had time to grow to maturity, recontaminate the pasture, and repeat the life cycle. The fact that Ransom was unable to maintain lambs free from *H. contortus* by the method reported in his paper throws some doubt on the conclusions drawn in connection with his survival studies.

An examination of reports of previous studies makes it possible to conclude only that larvae of *H. contortus* will survive over the winter under certain conditions, but the number of larvae surviving and capable of causing infection at any given time after pasture contamination is not definitely known. Therefore, the studies reported in this paper were made to ascertain whether enough larvae on a contaminated pasture die during the fall and winter to make the pasture safe for sheep the following spring. The work was carried on from August 1936 to March 1938 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

MATERIALS AND METHODS

In the present experiments 4-foot-square grass plots, each divided into 2-foot-square subplots, were used. The plots were separated by a space about 3 feet wide. The soil was sandy loam. The plots were sown with a mixture of bluegrass, meadow fescue, timothy, redtop, Italian rye, white clover, and alsike. Certain other plants appeared and were allowed to remain. As grasses were the most common plants present, the term "grass" is used as a designation for all the

vegetation. Except in the third experiment, the grass was cut at weekly intervals, if necessary, to keep it at a height of 4 inches. The grass trimmings were allowed to fall back on the plots, from which they disappeared shortly afterward.

The larvae and eggs used in the experiments were obtained from sheep infested either with *Haemonchus contortus* alone or with a combination of *H. contortus* and *Strongyloides papillosus*. Since the eggs and larvae of these two species can be easily distinguished, the slight contamination with *S. papillosus* may be ignored. No viable larvae of *S. papillosus* were found beyond the first week of exposure in any of the tests in which these larvae were originally present.

After the grass in the plots had grown to about 4 inches, feces from sheep infected with *H. contortus* were placed in the center of each subplot. In a week or more one of the subplots was examined for infective larvae, after which all the other subplots were examined at intervals of 1 or more weeks.

In examinations, the grass on a subplot was cut and the larvae washed from it. Larvae in feces, on the surface of the soil, and in the soil to a depth of 1 inch were recovered by means of the Baermann technique. In all cases the material studied—whether grass, feces, or soil—was taken from the entire subplot. The material was allowed to remain in the Baermann apparatus for 48 hours, and then about 30 cc. of water with any larvae present was withdrawn. If free-living nematodes were so numerous that they interfered with the counts of the larvae, the former were killed by means of hydrochloric acid by the method of Shorb (*13*). In each experiment, the difference between the average number of larvae in the control lots of feces and the number recovered on each date of collection represents the loss due to death of eggs and larvae as well as the loss due to manipulation of material. Loss of larvae in manipulation takes place in two ways. Some larvae migrate into the soil; others are lost in the Baermann apparatus, because recovery is never perfect.

Four experiments were conducted. In experiments 1 and 3, the parasitized feces were placed on the subplots in August and in experiments 2 and 4, in October.

WEATHER DATA

Development and survival of *Haemonchus contortus* larvae depend to a large degree on meteorological conditions. Death of eggs and preinfective larvae is caused by cold or drying; death of infective larvae, largely by drying. A summary of the weather data during the period in which the experiments were in progress is shown in table 1. During the first period of the first two experiments the weather was unusually dry at the beginning; during the third and fourth experiments it was unusually wet at the beginning. At other times the weather was usually not far from normal for the region where the experiments were conducted.

TABLE 1.—*Summary of weather conditions* [1] *during experiments*

Month	Temperature				Mean relative humidity at—			Precipitation	
	Maxi-mum	Mini-mum	Mean	Depar-ture from normal (mean)	8 a. m.	12 m.	8 p. m.	Total	Depar-ture from normal
1936	° *F.*	° *F.*	° *F.*	° *F.*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*
August	98	59	77.9	+2.9	77	56	66	3.61	−0.40
September	93	47	71.4	+3.3	78	58	69	1.98	−1.20
October	83	27	59.7	+2.3	81	55	68	1.70	−1.14
November	79	19	45.0	−.2	68	48	54	.76	−1.61
December	65	14	39.0	+3.2	78	62	68	5.28	+1.91
1937									
January	76	29	43.8	+10.4	79	75	73	7.83	+4.28
February	64	20	36.6	+1.3	70	54	56	3.33	+.06
March	70	19	42.0	−.6	68	47	50	1.50	−2.25
April	93	34	53.9	+.1	69	48	53	6.85	+3.57
May	91	40	65.8	−2.1	73	46	54	4.02	+.32
August	97	62	77.8	−2.8	86	62	72	6.70	+2.69
September	94	45	65.9	−2.2	85	55	69	1.76	−1.58
October	82	31	55.3	−2.1	82	57	69	8.81	+5.97
November	75	23	46.6	+1.4	73	46	56	3.88	+1.51
December	72	19	37.0	+.4	70	49	56	.71	−2.61
1938									
January	63	18	35.7	+2.3	74	59	61	2.64	−.91
February	71	21	40.9	+5.6	72	56	59	2.37	−.90
March	85	20	49.8	+7.2	72	55	58	2.23	−1.52

[1] Obtained from the monthly meteorological summary of the Washington, D. C., station of the Weather Bureau, U. S. Department of Commerce, located 14 miles from Beltsville, Md., where the experiments were carried out.

EXPERIMENT 1. LARVAE PLACED ON GRASS PLOTS AUGUST 11, 1936

For the first experiment, 16 subplots were prepared on August 11, 1936, by placing 100 gm. of crushed fresh sheep feces in a pile in the center of each subplot. Four 100-gm. cultures prepared from the same lot of feces yielded an average of 50,000 larvae, a fact which showed that the infective material was heavily parasitized. During the first 2 months grass only was examined. For the remainder of the test, grass and soil to the depth of 1 inch were examined. The average number of larvae recovered from each subplot and the date of collection are recorded in table 2. This experiment showed a survival of larvae for 9 months during summer, fall, winter, and spring. There was a gradual reduction in the number of larvae recovered. Those obtained in April and May were all vacuolated, and it is probable that they were not infective. The sudden increase of larvae recovered in March was noticed in subsequent experiments and was probably due to the greater warmth, which attracted the larvae to the surface of the soil.

TABLE 2.—*Number of Haemonchus contortus larvae recovered from 16 subplots on which fresh sheep feces were placed August 11, 1936*

Subplot No.	Date of collection	Larvae recovered [1]	Subplot No.	Date of collection	Larvae recovered [1]
	1936	*Number*		*1937*	
1a	Aug. 19	1,500	9d	Jan. 8	2
1b	Sept. 3	1,000	12c	Feb. 27	22
1c	Sept. 15	450	11a	March 24	278
1d	Sept. 22	397	11b	Apr. 3	108
12a	Oct. 15	700	11c	Apr. 20	3
9a	Oct. 28	96	11d	Apr. 27	28
12b	Dec. 16	133	12d	May 4	14
9b	Dec. 23	45			
9c	Dec. 30	46			

[1] From both grass and soil.

EXPERIMENT 2. LARVAE PLACED ON GRASS PLOTS OCTOBER 26, 1936

A second experiment was carried out in the same manner as the one just described except that it was begun in October instead of August and, because of unavoidable circumstances, 2 months elapsed before the first examination of plots instead of 1 week as in the first experiment. Four cultures prepared from the lot of feces placed on the plots yielded an average of 100,000 larvae. The average number of larvae recovered from each of 13 subplots is shown in table 3. In the first experiment a few larvae were alive after 9 months, but in experiment 2 all were dead in 7 months. It is not known definitely whether the surviving larvae were always capable of producing infection as no tests to determine their infectivity were made, but it is probable that they were not infective even as late as April as all larvae recovered at that time were feeble and vacuolated. As the subplots examined on May 15, 1937, had no larvae the 3 remaining subplots were not examined and consequently are not included in the table.

TABLE 3.—*Number of Haemonchus contortus larvae recovered from 13 subplots on which fresh sheep feces were placed October 26, 1936*

Subplot No.	Date of collection	Larvae recovered [1]	Subplot No.	Date of collection	Larvae recovered [1]
	1936	*Number*		*1937*	*Number*
5c	Dec. 21	118	6a	Mar. 8	112
5a	Dec. 30	182	4a	Mar. 24	48
	1937		7a	Apr. 20	8
			4d	Apr. 27	3
5d	Jan. 8	20	4c	May 15	0
5b	Jan. 15	112	6c	May 15	0
4b	Jan. 22	116	6d	May 15	0
6b	Feb. 24	64			

[1] From both grass and soil.

EXPERIMENT 3. LARVAE PLACED ON GRASS PLOTS AUGUST 5, 1937

The third experiment was initiated on August 5, 1937, by placing 50 gm. of crushed feces in the center of 12 subplots. Although the 50-gm. control cultures yielded an average of 10,640 larvae, the grass of a subplot examined on August 12, 8 days after the experiment was begun, yielded only 463 larvae. Soil and grass of a second subplot, examined on August 20, 1937, yielded 1,364 larvae. After the apparently rapid loss of larvae or eggs of *Haemonchus contortus*, as shown by the examination of August 12, it was believed that there were too few larvae left for the purposes of the experiment. Therefore, on August 20 additional larvae were placed on each remaining subplot in 70 gm. of feces that had been cultured for 4 days. An average of 27,265 larvae were recovered from 4 samples of 70 gm. each of the same lot of feces used on these plots. In this experiment the grass was not trimmed to keep it at a length of 4 inches. At intervals of about 1 week, soil and grass were removed from the subplots and examined, with the results shown in table 4. Except for collections made on October 2, the reduction in numbers of larvae recovered from the plots was constant and rapid. Larvae were found at all times not only on the surface of the soil, but also under the surface and on the grass.

TABLE 4.—*Number of Haemonchus contortus larvae recovered from grass and soil of subplots infected on August 5 and on August 20, 1937*

| Subplot No. | Date of collection | Larvae recovered from— | | | Total larvae recovered |
		Grass	Surface of soil	Soil to 1 inch deep	
		Number	*Number*	*Number*	*Number*
12a	Aug. 28	5,904	30	106	6,040
12b	Sept. 9	4,606	342	850	5,798
11d	Sept. 17	2,749	180	--------	2,929
11c	Sept. 23	1,402	63	167	1,632
11b	Oct. 2	3,944	576	1,920	6,440
11a	Oct. 9	784	50	24	968
7d	Oct. 17	122	21	100	243
7c	Oct. 30	18	1	5	24
7a	Nov. 6	22	4	29	55
7b	Nov. 18	--------	2	26	49

EXPERIMENT 4. LARVAE PLACED ON GRASS PLOTS OCTOBER 11, 1937

In the first three experiments eggs and larvae were exposed to natural conditions, and the survivors were recovered from the plots as infective larvae. In the fourth experiment, to simplify the problem by eliminating consideration of survival of the eggs and preinfective stages, the larvae were kept in the laboratory until they had reached the infective stage and then were placed on the grass plots. For this study, infective larvae in 75 gm. of crushed feces were placed in the center of 16 subplots on October 11, 1937. Four lots of 75 gm. of feces each yielded an average of 38,500 larvae. Collections of material from the subplots were made at approximately weekly intervals. No samples of soil were taken from December to March while the ground was frozen. In the next to the last column of the table, the result shown for March 24 is for a pooled sample of the subplots indicated. The soil was removed in 1-inch layers to a depth of 3 inches, each inch of soil being examined for larvae separately. Four larvae were recovered from the 2-inch level and 5 from the 3-inch level. The number of larvae recovered from other samples is shown in table 5.

TABLE 5.—*Number of Haemonchus contortus larvae recovered from grass and soil of subplots receiving infective larvae on October 11, 1937*

| Subplot No. | Date of collection | Larvae recovered from— | | | | Total larvae |
		Grass	Feces	Soil surface	Soil 1 inch deep	
	1937	*Number*	*Number*	*Number*	*Number*	*Number*
1a	Oct. 18	4,944	7,633	4,761	2,880	20,218
1b	Oct. 25	780	24	180	47	1,031
1c	Nov. 1	1,944	555	104	48	2,651
1d	Nov. 8	1,848	480	540	42	2,910
2a	Nov. 15	252	80	325	122	779
2b	Nov. 21	284	16	68	3	371
2c	Nov. 29	705	225	45	23	998
2d	Dec. 6	1,360	76	30	(1)	1,472
	1938					
3a	Jan. 15	140	83	(1)	--------	222
3b	Feb 6	112	26	(1)	--------	138
3c	Feb. 19	34	32	(1)	--------	66
3d	Mar. 5	35	4	(1)	--------	39
4a	Mar. 19	90	129	(1)	--------	319
4b	Mar. 24	22	9	31	}	[3] 62
4c	do	37	8	29	[2] 7	[3] 64
4d	do	24	9	31	}	[3] 64

[1] No samples taken as ground was frozen.
[2] Recovered from a pooled sample of soil from all 3 plots removed to a depth of 1 inch.
[3] Does not include soil sample 1 inch deep.

The reason for the low recovery of larvae in the latter part of October and the high recovery in the latter part of November and the first part of December is not known. The decrease in number of larvae recovered on the other dates indicates that death was constant and rapid. Here, too, as in experiment 3, larvae were always recovered from the grass, from the soil surface, and beneath the soil surface whenever the examination was made. There was an increase in number of larvae recovered on March 19, similar to the finding in experiment 1. In experiment 4, in which larvae were placed on the plots, 52.5 percent were recovered 7 days later; in experiment 1, in which eggs were used, only 3 percent were recovered as larvae 8 days later. The difference between 52.5 and 3 percent indicates a high death rate among eggs and preinfective larvae and a much lower death rate of infective larvae.

SUMMARY AND CONCLUSIONS

Experiments were conducted from August 1936 to March 1938 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., to determine how long eggs and larvae of the large stomach worm (*Haemonchus contortus*) would survive under outdoor conditions during different seasons of the year. Feces of sheep infected with the parasite were placed on 2-foot-square grass plots in August and October.

During the fall and winter, infective larvae were recovered from the plots but in much smaller numbers than during the summer or early fall. In each of three tests continued until spring, the number of larvae recovered in March was higher than in the preceding month. This result probably was due to larvae being attracted to the surface of the soil by rising temperatures. A few infective larvae exposed on grass plots in August or October survived until the following spring, but all larvae surviving until April or May were sluggish and vacuolated and probably noninfective.

These findings indicate that pastures kept free of sheep from October until the middle of April will contain only a few larvae of *H. contortus* still capable of infecting these host animals.

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FACTORS AFFECTING THE ASCORBIC ACID CONTENT OF CABBAGE LINES[1]

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INTRODUCTION

Recent summaries of quantitative estimates of vitamin content of plant and animal foods give bewildering ranges in the readings. It has been shown that concentration of the same vitamin varies from tissue to tissue in the same organ (*3*),[2] from fruit to fruit on the same tree (*6*), from season to season on the same variety (*5*), and, of especial interest to the plant breeder, from variety to variety (*2*). A study of ascorbic acid content in cabbage from Ohio (*2*) showed that among 30 varieties grown there in the summers of 1938 and 1939 there were significant differences with average vitamin C content for 2 head samples each ranging from 0.48 mg. per gram of fresh weight for the variety All Head Select to 1.81 for Midseason Market, 2 varieties having practically the same average weight. This high reading of head leaves of the latter exceeds the previously recorded maximum of 1.58 mg. per gram for head leaves reported in 1939 from Texas (*4*); it places maximum readings for cabbage ahead of those for citrus juices, tomatoes, and strawberries as sources of ascorbic acid in the American dietary (*3*).

MATERIALS AND METHODS

A study of some factors determining variability in ascorbic acid content in cabbage was conducted with 25 breeding lines planted in a randomized-block design having 3 replicates in the fall of 1941 and 3 in the spring of 1942. The randomized-block design permitted study of the variability of ascorbic acid readings with and without the correlated effect of head weight.

Variance of ascorbic acid alone was compared with the covariance of ascorbic acid on head weight; and covariance was found as the errors of estimate when the main terms were compared by Snedecor's method (*9, pp. 249–273*) with the error term. The mean unadjusted ascorbic acid readings were adjusted by the following formula:

$$X = Y - bx$$

where Y = individual ascorbic acid readings
b = regression coefficient of the error term
x = departure of weight from the general mean
X = adjusted individual ascorbic acid reading.

The breeding lines selected for this study are shown in table 1. All lines were obtained from survivors of the low temperatures of the fall of 1938.

Head characters were classified in the field at the time of harvest, and ascorbic acid content was obtained as soon as possible afterward by Morell's (8) rapid adaptation of the Bessey (1) and Mindlin and Butler (7) procedure. Samples were obtained by cutting the heads through the axis, removing an aliquot segment of each carefully with a large knife, and then macerating the tissue as described by Morell (8).

TABLE 1.—*Mean weight and ascorbic acid readings for 25 breeding lines of cabbage grown in 2 seasons, fall 1941 and spring 1942, together with adjusted ascorbic acid values*

Breeding lines	Head weight		Ascorbic acid per gram				Ranking of adjusted values	
			Unadjusted		Adjusted [1]			
	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring
	Pounds	*Pounds*	*Milligram*	*Milligram*	*Milligram*	*Milligram*		
Self-pollinated Volga:								
534–5–1–3	3.7	2.4	0.50	0.50	0.48	0.49	21	21
534–5–1–4	3.3	1.9	.54	.54	.52	.49	12	21
534–5–3–1	3.9	1.6	.50	.66	.51	.59	14	6
534–5–5–1	2.3	2.2	.36	.51	.31*	.48	25	23
534–7–4–2	3.7	2.9	.51	.46	.51	.48	14	23
534–15–4–1	3.6	2.5	.56	.52	.56	.51	7	18
534–16–2–1	3.4	2.3	.58	.50	.57	.48	4	23
Open-pollinated Charleston Wakefield (F₃):								
543–0₁–3–1	1.9	1.5	.80	.68	.73*	.61	1	2
543–0₁–3–4	2.9	1.7	.52	.67	.49*	.61	20	2
543–0₁–3–29	3.5	3.4	.58	.54	.57	.59	4	6
543–0₁–3–40	3.2	1.6	.62	.58	.60	.51	2	18
543–0₁–3–47	3.0	2.1	.53	.64	.50*	.61	18	2
543–0₁–5–3	2.7	2.5	.54	.54	.50	.53	18	16
543–0₁–5–12	1.9	2.1	.67	.60	.60	.57	2	10
543–0₁–7–4	4.9	2.8	.52	.57	.57	.58	4	9
543–0₁–7–7	5.3	3.3	.45	.54	.51	.59	14	6
543–0₁–11–19	4.9	2.8	.43	.55	.48	.56	21	11
543–0₁–11–23	3.8	2.8	.56	.52	.56	.53	7	16
543–0₁–11–26	5.3	3.4	.46	.49	.52	.54	12	13
543–0₁–11–32	4.2	3.4	.53	.55	.55	.60	9	5
Controlled cross (Volga×Charleston Wakefield):								
BCC 2–1–10 (F₃)	3.8	2.6	.44	.55	.44*	.55	24	12
Open-pollinated All Head Early:								
537–0₁–1–3	3.9	2.9	.47	.49	.48	.51	21	18
All Head Select:								
539–0₁–1–1	5.1	2.4	.48	.55	.53	.54	11	13
539–0₁–5–3	3.5	2.7	.52	.53	.51	.54	14	13
Wisconsin Ballhead:								
550–0₁–9–1	3.8	2.9	.55	.64	.55*	.66	9	1
General mean	3.70	2.57	.529	.553	.530	.564		

[1] * indicates that fall and spring values were significantly different. A difference of 0.11 mg. was necessary for significance at 5-percent point.

RESULTS

Table 1 gives the mean head weights and the unadjusted and adjusted ascorbic acid readings for 25 breeding lines. The smaller average head weight of the spring crop, 2.57 ± 0.07 pounds, compared with that of the fall crop, 3.70 ± 0.10 pounds, was accompanied by a higher average ascorbic acid content, 0.553 ± 0.007 mg. In the fall the average content was 0.529 ± 0.007 mg. The differences between general means for the two variables are statistically significant at the 1-percent point, 1.13 ± 0.12 pounds for weight and 0.024 ± 0.009 mg. for ascorbic acid.

Table 2 shows F values for the variance in ascorbic acid and for the covariance of ascorbic acid on head weight for the two seasons singly

and combined. The standard error of a single determination, 0.067 in the fall and 0.062 in the spring, and the correlations between weight of head and ascorbic acid, viz -0.55**[3] in fall and -0.61** in spring for error sources of variance, are practically the same. Variance for lines is highly significant, but that for replicates is not significant.

When seasons are combined, there is a highly significant seasonal effect in the variance of ascorbic acid, 7.88**, but when regression on weight is considered as in the covariance of season compared with error, the significance is eliminated, 2.97. The significance of the variety term in variance, 5.90**, and covariance, 5.51**, is above the 1-percent point. Ascorbic acid readings are adjusted to the regression data for general mean head weight in columns 6 and 7 of table 1.

TABLE 2.—*Analysis of variance and regression data for values in table 1*

Sources of variability	Variance		Regression data			
	Degrees of freedom	F values [1]	Degrees of freedom	F values for covariance [2]	Regression coefficient	Correlation coefficient
Fall 1941:						
Lines	24	5.04**		5.22**		
Replicates	2	.84		.22		
Error			47		−0.0378	−0.5527**
Total			73		−.0434	−.5282**
Spring 1942:						
Lines	24	2.85**		2.79**		
Replicates	2	2.15		1.25		
Error			47		−.0669	−.6144**
Total			73		−.0695	−.6348**
Both seasons:						
Lines	24	5.90**		5.51**		
Replicates [3]	4	1.45		.97		
Season	1	7.88**		2.97		
Seasons × lines [2]	24	2.14**		2.59**		
Error			95		−.0453	−.5607**
Total			148		−.0448	−.5597**

[1] Standard errors for a single determination are fall, 0.067; spring, 0.062; both, 0.065.
[2] F values for covariance when the interaction term is used instead of the error term are for lines 2.06*, and for season, 0.53.
[3] Variability of replicates within seasons.
**=Significant at the 1-percent point.

Inconsistency in behavior of these 25 lines in the 2 seasons is shown by the F value of 2.06* for the comparison (footnote 2, table 2) of the covariance for lines with the interaction term. The details of the inconsistency are seen in the columns of table 1 giving mean unadjusted and adjusted ascorbic acid values. Furthermore, the coefficient of correlation between 1941 and 1942 unadjusted mean readings is 0.4616*, whereas between the adjusted readings it is 0.3353.

DISCUSSION

Table 2 shows a highly significant degree of negative correlation between ascorbic acid and head weight, $-.5607$**, for the combined-seasons error term; this is low enough, however, to indicate that head weight is not a major factor in affecting concentration of ascorbic acid.

The relative importance of heredity (line sources of variability) and environment (replicates and seasons) in producing ascorbic acid

* Values marked ** are statistically significant at the 1-percent point; those marked * at the 5-percent point.

differences between breeding lines is shown in table 2 by the relative sizes of F values for variance and covariance analyses. Column 3 shows these relative values when the effect of head weight is not eliminated (variance), and column 5 when it is (covariance).

The F values for variance suggest that seasonal effect, 7.88**, is more important than line effect, 5.90**, and that both effects are significant at the 1-percent point, whereas no significance is attached to replicates (soil variability). The F values for covariance, where head weight influence is eliminated, show highly significant line variability, but nonsignificant seasonal variability. In other words, variability in ascorbic acid between breeding lines is determined mainly by hereditary factors, and seasonal influence operates mainly on head size and therefore to a lesser degree on ascorbic acid content.

Comparison of the covariance for lines with that of the interaction term discloses a significant degree of inconsistent varietal (line) behavior from season to season. The extent of inconsistency is shown after adjustment of the ascorbic acid readings according to the regression coefficients for the two seasons in the last four columns of table 1. The new values in ascorbic acid content are best shown by their relative rankings.

There is greater variability in ascorbic acid in the fall than in the spring, and some lines apparently do better in one season than another. Application of the size of a significant difference between seasons, 0.11 mg., to the data for adjusted mean readings in ascorbic acid discloses six significant differences. Most, but not all, of these differences favor the spring. The outstanding exception is line 543-0_1-3-1, which, although always near the lead, apparently does significantly better in the fall.

As far as temperature is related to ascorbic acid there appears to be evidence that the ultimate effect, whether enhancing or depressing, depends on the genetic constitution of the line in question.

A noteworthy line is 550-0_1-9-1, which in addition to being consistently high in ascorbic acid, is also above average in head weight and serves, with lines 543-0_1-7-4 and 543-0_1-11-32, to demonstrate that small weight is not a primary factor in producing high ascorbic acid.

SUMMARY

There is a significant degree of negative correlation between ascorbic acid concentration and head weight among 25 breeding lines of cabbage grown as 3 replicates in the fall and 3 in the spring.

Of the six lines with more consistently high ascorbic acid content, judged by adjusted ranking, three were above average in head weight.

Regression data show that hereditary differences are more important than seasonal or positional differences in producing ascorbic acid differences between breeding lines.

Seasonal effect on ascorbic acid is expressed chiefly on head size and secondarily on genetic constitution.

Only six lines differed significantly in ascorbic acid content from season to season. In only one of these was the content higher in the fall than in the spring.

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A COMPARISON OF THE VISCOSITY AND CERTAIN MICROSCOPICAL PROPERTIES OF SOME KANSAS STARCHES [1]

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INTRODUCTION

The investigation herein reported represents the continuation of an effort to determine the practicability of utilizing Kansas-grown potatoes and sweetpotatoes as starch crops. It has been found previously (1)[2] that Kansas potatoes compare favorably with those of other areas in the quantity of the starch they contain. There remained the problem of evaluating them with respect to the quality of the starch.

A partial answer to the practicability of producing starch from the crops of any area is determined by the suitability of the derived starch for nonfood uses, such as pastes, sizings, and mucilages. Accordingly, measurements were made of the physical behavior which may be affected by the granule size, granule size distribution, and the nature of the granule.

The gelatinization temperature is a property of practical importance to starch manufacturers in that it reflects in some measure the suitability of a starch for a given purpose. According to Radley (11), there is a close connection between the granule size and the gelatinization temperature, as shown by the fact that, in the same starch suspension, the larger granules appear to swell at a lower temperature than the smaller ones. Furthermore, other factors being equal, starch with an average size smaller than normal will require a somewhat higher temperature and more heat for the conversion to dextrin.

A marked variability is frequently observed in the viscosity of starch pastes at corresponding stages of development. Szego (14) and Janicki (6) state that the viscosity of a paste is directly related to the average size of the granules, the smaller granules producing a more viscous paste. Caesar and Moore (3) have shown that each type of starch will give a characteristic viscosity record, information that is useful in determining the usage of a particular starch. Thus, the individual differences which govern the usefulness of a starch appear to be associated in some way with the permeability and physical structure of the starch granule as well as with the granule size.

The object of this investigation, therefore, was the measurement of the granule size, granule size distribution, gelatinization temperature, and the viscosity of native potato and sweetpotato starches for the purpose of their comparison with each other and with commercial domestic starches and, further, to learn of the effects of time of harvest and cure on the quality of starch.

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EXPERIMENTAL PROCEDURE

TREATMENT OF SAMPLES

The potatoes (*Solanum tuberosum L.*) and sweetpotatoes (*Ipomoea batatas* (*L.*) *Lam.*) used in this investigation were listed and described in an earlier paper (*1*). The samples were obtained from test plots distributed in the potato-growing areas of the State. For their production, proper regard for agronomic procedure was exercised in the selection of soil types, preparation of plot areas, dates of planting and harvesting, cultivation and general care.

Immediately upon arrival of the potatoes, the starch was extracted from all samples except those that were stored or cured, in which cases the starch was not removed until the end of the process. The potatoes were ground in a large meat grinder and sufficient sodium bisulfite was added to give a concentration of 0.15 percent. The ground material was passed through the grinder three additional times, after which the starch was worked out through muslin cloth, washed repeatedly by suspending it in a 0.15-percent soldium bisulfite solution, and allowed to settle before the supernatant liquid was drawn off. After washing the suspension in this manner until the bulk of foreign matter had been removed, and then sieving, the starches were separated by tabling and most of the water was removed on a Büchner funnel under gentle suction. On the following day the starches were air-dried, weighed, and stored in glass containers. Starches thus obtained were white, odorless, and contained practically no foreign matter such as dirt, cellulose, or pigmented granules, that could be detected with a microscope.

DETERMINATION OF THE GELATINIZATION TEMPERATURE

There are several methods of determining the gelatinization temperature. Küntzel and Doehner (*7*) developed a method based on the change in light absorption during gelatinization. Ceasar and Moore (*3*) considered the gelatinization temperature as the range between the temperature at which the viscosity first becomes measurable and that which corresponds to the initial maximum. McNair (*10*), Reichert (*12*), and Francis and Smith (*4*) used that property which the granules possess of losing their polarization crosses when they start to gelatinize. Other methods depend upon the development of a color change in stained granules, a difference in solubility of the granules after gelatinization, and a difference in the volume at the gelatinization temperature. Principally because the gelatinization temperatures of the majority of starches have been based upon the loss of anisotropy, the method of Francis and Smith was selected for this work.

The thermoslide of the gelatinization temperature measuring device was provided with two thermometers for measuring the inlet and outlet temperatures of the heating fluid; the average of these temperatures was taken as the temperature of the starch under observation. The validity of the average temperature was tested with *p*-bromobenzene, whose melting point of 87°–88° C. is not far removed from the temperature range used. The average of several melting-point determinations made on the thermoslide was 87.8°.

In making a determination, two drops of distilled water were placed in the exact center of the upper window, the surface of the water sprinkled with starch, and the dilute suspension covered with a thin cover glass. The microscope was focused upon the suspension and the examination was made with a 4-mm. objective. By the proper adjustment of the polarizer and analyzer with respect to each other, the granules appeared as small bright crosses on a dark background. The fading out of these crosses with the rising temperature marked the disappearance of anisotropy from the granules. The temperature was raised at a rate of 8° C. per minute and the temperatures were taken when the polarization crosses began to fade away regularly and again when nearly all the crosses had faded away. In both potato and sweetpotato starches there were nearly always a few granules the anisotropy of which was lost at an abnormally low temperature and also some whose anisotropy persisted to an extent that, if they were considered, the limits of the gelatinization temperature would be meaningless. Wherever reference is made in the text to the gelatinization and mean gelatinization temperatures, the former is regarded as the temperature range through which the granules lose their anisotropy and the latter as the average of the extreme temperatures of this range.

DETERMINATION OF GRANULE SIZE AND GRANULE SIZE DISTRIBUTION

To determine the granule size of the starch particles, the long axis was measured with a calibrated ocular micrometer on a microscope as described by Garner (5). In order to obtain a uniformly dilute sample to observe, a small quantity of starch was placed in two drops of glycerol on a black glass plate and mixed thoroughly; a very small portion of this was placed on the slide with the addition of another drop of glycerol and covered with a cover glass. In this manner 500 granules from a uniformly dilute mixture were measured. To check upon the reliability of this method a count of 5,000 granules was made from a sample, and the results showed that a count of 500 was representative. Lindet and Nottin (9) state that a starch can be characterized with sufficient accuracy if 150 to 200 granules are measured, but it was found in this study that a count of less than 500 does not always permit a satisfactory statistical treatment.

DETERMINATION OF VISCOSITY

The viscosity of the starch pastes was measured in a viscometer designed by Barham and Reed; the description and calibration of the instrument, together with the detailed method of making determinations, are given by Barham, Wagoner, and Reed (2).

The viscosity is measured by the weight in grams that must be applied to prevent the rotation of a cylinder suspended in a rotating cup containing the starch paste. This relationship is defined by the equation

$$\frac{W}{\eta\,l}=k$$

in which W represents the weight in grams, η the absolute viscosity. l the height of the cylinder wall exposed to the action of the liquid, and k the instrument constant which in this case has a value of 0.79,

In making determinations, the starches were made into 10-percent "starch milks" which were heated slowly to 93°–94° C. After cooking the resulting pastes at 93° for one-half hour, they were cooled slowly to 35°–40°. Throughout the temperature cycle, temperature and viscosity readings were made at regular intervals.

RESULTS

VISCOSITY

Viscosity determinations were made on all samples prepared in the laboratory, and table 1 gives a representative sample of the data
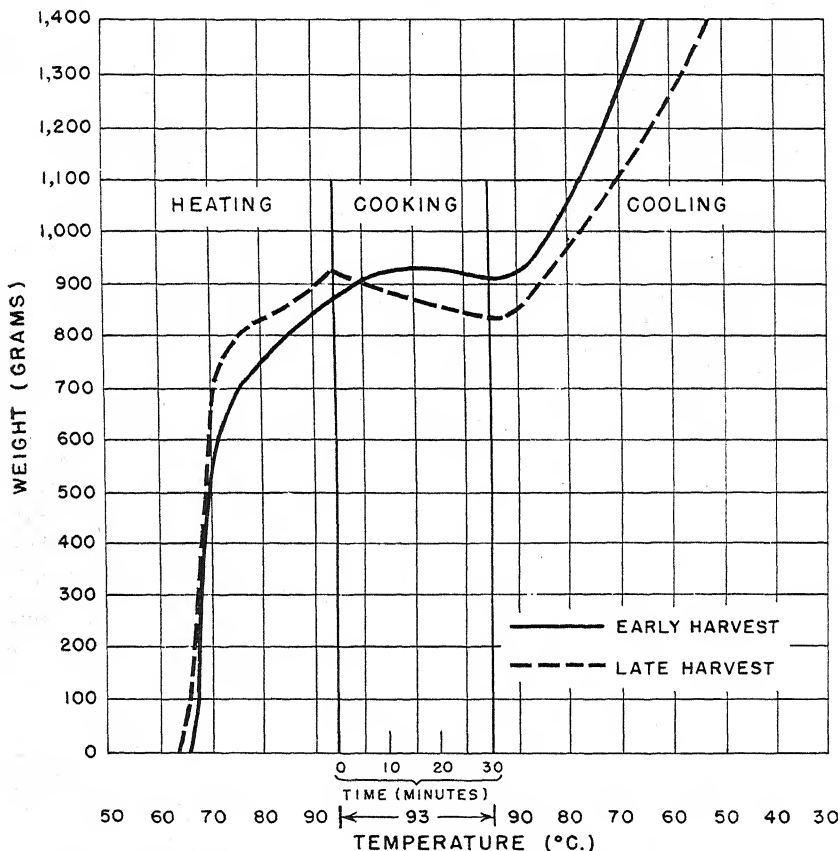
FIGURE 1.—The effect of time of harvest on the viscosity of the starch from potatoes (Warba variety).

taken. In each determination data were taken each minute until the cooking period was reached after which readings were taken at 5-minute intervals; but to shorten the table the data are listed for only 5-minute intervals. From such data smooth curves result when the weight in grams (W), as measured by the balance, is plotted against the temperature.

The effects of early and late harvest upon the starch from the Warba variety of potatoes are shown in figure 1.   The curves in this figure are representative of all three varieties tested; in every case the late-harvest potatoes formed a starch that had a lower temperature at the first observable change in viscosity, thinned out more dur-
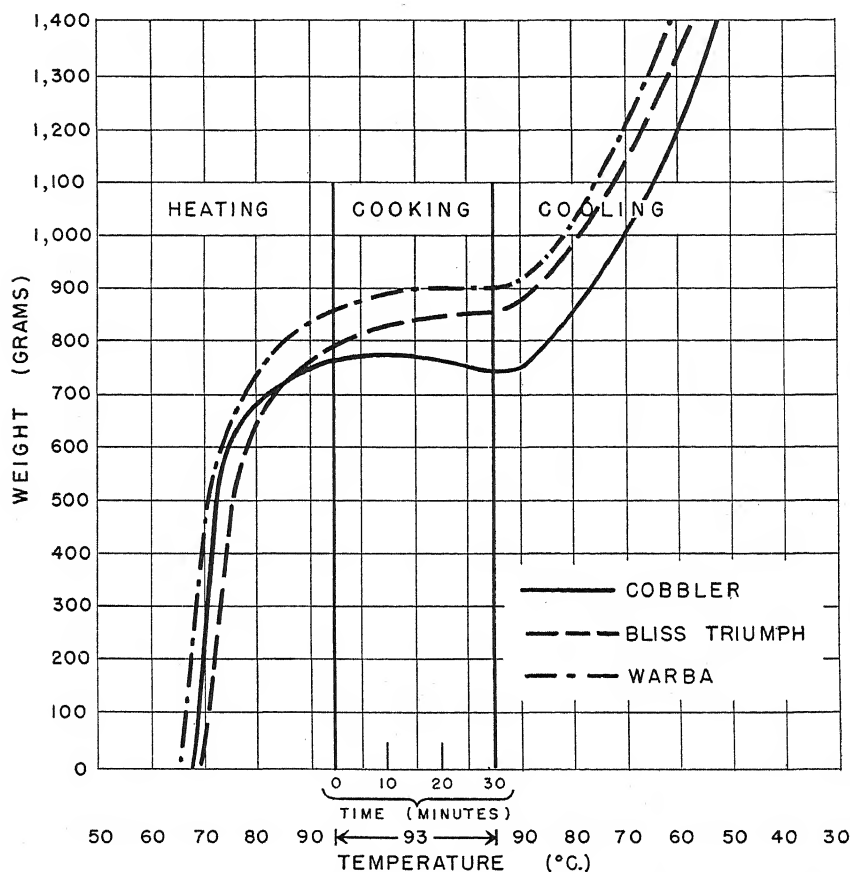
FIGURE 2.—Comparison of the viscosity of starch from three common varieties of potato, indicating the effect of variety.

ing the cooking, and never attained the maximum viscosity of the starch from early potatoes.

In figure 2 there is a comparison of starches of the three common varieties of potatoes. This shows that the Warba exhibited the lowest temperature at which the viscosity curve starts, the highest maximum, and the most viscous paste when cooled. The Bliss Triumph showed a higher temperature than Irish Cobbler for the initial part of the viscosity curve, but the Cobbler thinned out more on cooking and did not give so viscous a paste on cooling.

TABLE 1.—*Viscosity record of a 10-percent suspension of late-harvest Bliss Triumph (sample 15)*

Time	Oil-bath temperature	Paste temperature	Temperature lag	Weight (W)	Time	Oil-bath temperature	Paste temperature	Temperature lag	Weight (W)
	°C.	°C.	°C.	Grams		°C.	°C.	°C.	Grams
1:30	69.0	61.0	8.0	------	2:40	97.0	92.0	5.0	803
1:35	70.5	65.0	5.5	------	2:45	98.0	93.0	5.0	798
1:40	73.0	67.7	5.3	------	2:50	97.0	92.7	4.3	795
1:45	75.5	70.5	5.0	20	2:55	97.0	92.2	4.8	795
1:50	78.0	71.7	6.3	255	3:00	88.0	88.0	0	830
1:55	80.0	73.5	6.5	535	3:05	74.0	80.0	6.0	940
2:00	81.0	76.0	5.0	612	3:10	70.0	76.0	6.0	990
2:05	83.5	77.7	5.8	626	3:15	64.0	71.0	7.0	1,090
2:10	86.0	80.5	5.5	654	3:20	60.0	66.0	6.0	1,190
2:15	92.0	84.0	7.0	680	3:25	54.0	61.0	7.0	1,295
2:20	96.0	89.0	7.0	703	3:30	48.0	56.0	8.0	1,440
2:25	98.0	92.0	6.0	750	3:35	43.0	49.0	6.0	1,610
2:30	97.0	92.5	4.5	790	3:40	39.0	45.0	6.0	1,750
2:35	96.0	92.0	4.0	810					
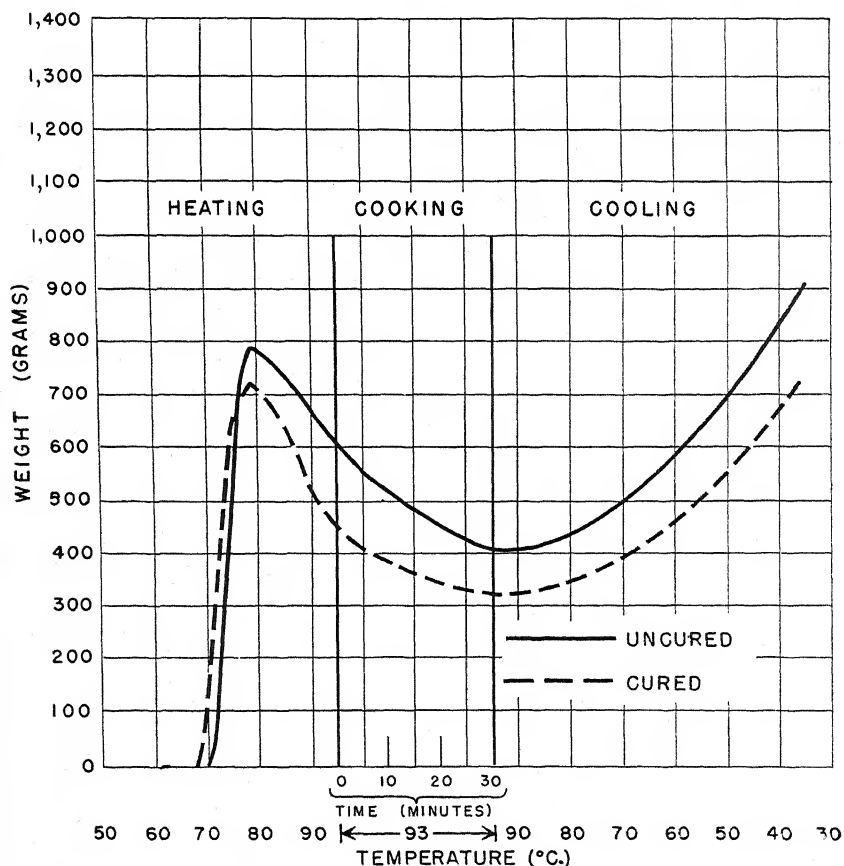
FIGURE 3.—The effect of curing on the viscosity of starch from sweetpotatoes.

Figure 3 represents the effect of curing on the sweetpotato starches. it is to be noted from figure 3, which is typical of all the starches examined, that the starch from the cured sweetpotatoes had different properties from that of the uncured.   This starch showed the initial viscosity change at a lower temperature, did not become so viscous, and thinned out more than the starch from the uncured sweetpotatoes. Figure 4 is a viscosity graph of the five varieties of sweetpotatoes which, with the exception of the Big Stem Jersey variety, were grown in the same plots and under similar conditions.   The Little Stem
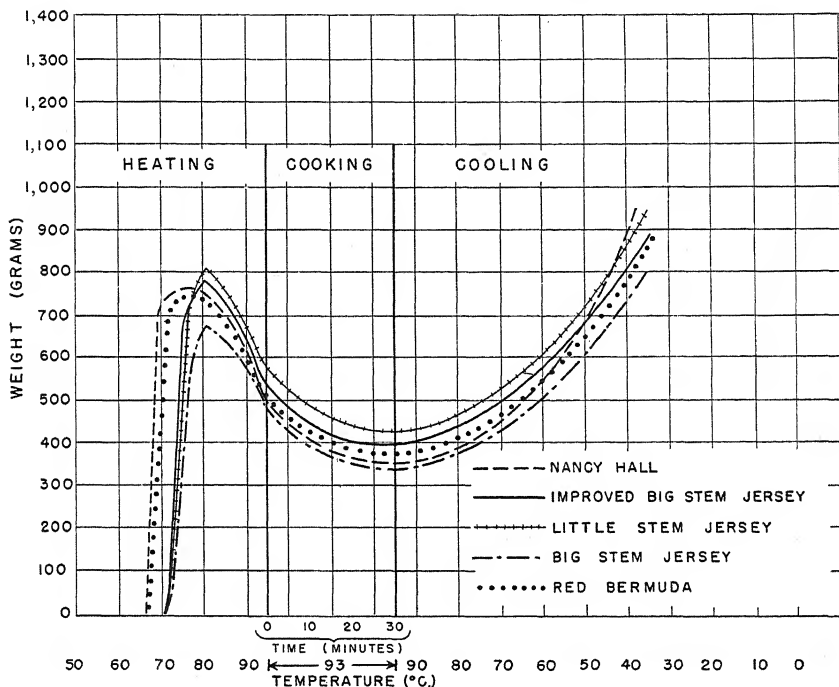
FIGURE 4.—Comparison of the viscosity of starch from five varieties of sweetpotatoes, indicating the effect of variety.

Jersey formed the most viscous paste with the least thinning out, while the Big Stem Jersey formed the least viscous paste with the most thinning out.   In general the shapes of the viscosity curves of the five varieties are about the same, although viscosity of the Nancy Hall and Red Bermuda varieties developed at lower temperatures and continued over a wider temperature range during the heating stage.

GELATINIZATION TEMPERATURE AND AVERAGE DIAMETER OF GRANULES

The results of the gelatinization temperature and the average diameter measurements of starches are given in tables 2 and 3. Usually in the literature only one temperature is given for sweetpotato or potato starch, and more frequently no mention is made of the particular variety upon which the determination was made.

But in this study it is shown that there were varietal and environmental variations with respect to gelatinization temperature and granule size for potatoes and sweetpotatoes. The variation in the extremes of the gelatinization temperature was from 4.5°–6.0° C. for potato and 5.5°–10.0° for sweetpotato starches. A statistical analysis [3] of these data shows that there are significant varietal differences in the gelatinization temperature.

TABLE 2.—*Effects of variety and curing on the gelatinization temperature, mean gelatinization temperature, and average granule diameter of sweetpotato starches, and a comparison of these starches with starch from a commercial source*

Sample No.	Source of starch	Treatment	Gelatinization temperature	Mean gelatinization temperature	Average diameter of granules
			° C.	° C.	*Microns*
5	Little Stem Jersey	None	70. 5–77. 0	74. 0	9. 10
16	----do	Cured	68. 5–77. 5	73. 0	8. 27
12	Red Bermuda	None	66. 5–76. 5	71. 5	9. 50
14	----do	Cured	66. 0–72. 5	69. 0	8. 96
6	Improved Big Stem Jersey	None	70. 0–79. 5	75. 0	9. 78
15	----do	Cured	68. 0–78. 0	73. 0	8. 69
10	Big Stem Jersey	None	70. 5–80. 5	75. 5	9. 12
13	Nancy Hall	None	67. 0–72. 5	70. 0	8. 49
(¹)	Stein, Hall & Co		70. 0–76. 0	73. 0	11. 41

[1] Domestic commercial starch included for comparison.

TABLE 3.—*Effects of variety and time of harvest on the gelatinization temperature, mean gelatinization temperature, and average granule diameter of potato starches, and a comparison of these starches with starch from a commercial source*

Sample No.	Source of starch	Time of harvest	Gelatinization temperature	Mean gelatinization temperature	Average diameter of granules
			° C.	° C.	*Microns*
1	Irish Cobbler	Early	67. 0–73. 0	70. 0	23. 67
8	----do	Late	69. 0–75. 0	72. 0	26. 06
4	----do	Early	66. 5–72. 5	69. 5	24. 26
16	----do	Late	67. 0–73. 0	70. 0	26. 08
2	Bliss Triumph	Early	66. 5–71. 5	69. 0	22. 64
15	----do	Late	70. 0–75. 0	72. 5	22. 40
3	Warba	Early	70. 0–74. 5	72. 5	33. 91
14	----do	Late	69. 5–75. 0	72. 5	28. 28
(¹)	Stein, Hall & Co		59. 5–68. 5	64. 0	29. 06

[1] Domestic commercial starch included for comparison.

Table 2 also illustrates the effect of curing upon the gelatinization temperature and granule size. In the curing process, which is done to heal the breaks in sweetpotatoes and to give a sweeter taste, the sweetpotatoes were heated at 26.7°–32.2° C. for 10 days to 2 weeks. The curing had the effect of lowering the gelatinization temperature and reducing the granule size.

Table 3 summarizes data showing the effects of the time of harvest upon the gelatinization temperature and the granule size of potato starches. These data indicate that the later harvest raised the gelatinization temperature for the Irish Cobbler and Bliss Triumph

(for Warba there was no change), raised the average granule size for the Irish Cobbler, and lowered the average granule size for the Warba and Bliss Triumph.

GRANULE SIZE DISTRIBUTION

When the granule sizes of the starches were arranged as to their frequency, it was found that both the potato and the sweetpotato starches were quite regular, with a majority of the granules about halfway between the two limits. Table 4 gives a representative sample of the granule size and distribution for a potato starch. In table 5, the percentage of granules occurring in each size range is given for the sweetpotato starches; similar data are given in table 6 for potato starches. It is evident that the effects of variety and curing on sweetpotato starches and of variety and time of harvest on potato starches showed different ranges and distribution of sizes.

TABLE 4.—*Data obtained in the size-frequency determinations of potato starch (sample 1)*

Step interval (microns)	Mid-point	Frequency		Step interval (microns)	Mid-point	Frequency	
	Mi-crons	*Num-ber* [1]	*Percent*		*Mi-crons*	*Num-ber* [1]	*Percent*
2–6	4	0	0	38–42	40	23	4.6
6–10	8	24	4.8	42–46	44	15	3.0
10–14	12	64	12.8	46–50	48	13	2.6
14–18	16	87	17.4	50–54	52	3	.6
18–22	20	102	20.4	54–58	56	5	1.0
22–26	24	67	13.4	58–62	60	3	.6
26–30	28	40	8.0	62–66	64	1	.2
30–34	32	27	5.4	66–70	68	1	.2
34–38	36	25	4.0	70–74	72	0	0

[1] Total=500.

TABLE 5.—*The percentage of granules occurring in each size range for some sweet-potato starches*

Midpoint (microns)	Improved Big Stem Jersey		Little Stem Jersey, uncured	Big Stem Jersey, uncured	Red Bermuda uncured	Nancy Hall, uncured
	Uncured	Cured				
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0						
2		0.4			1.0	2.6
4	7.2	8.8	7.0	4.8	8.4	6.8
6	15.8	18.6	18.0	19.2	11.4	21.8
8	25.0	40.8	30.6	20.8	32.4	34.4
10	19.8	10.0	21.0	21.6	17.4	16.4
12	15.4	12.6	11.4	14.6	15.4	10.8
14	7.0	4.0	6.6	4.4	5.6	3.0
16	5.8	2.8	3.8	2.8	4.2	2.0
18	1.4	.2	1.2	1.2	2.0	.6
20	1.8	1.0	.4	.4	1.8	1.6
22	.2	0		.2	.2	
24	.2	.6				
26	.4	.2				
28						
30						
Average diameter	*Microns* 9.78	*Microns* 8.69	*Microns* 9.10	*Microns* 9.12	*Microns* 9.50	*Microns* 8.49

TABLE 6.—*The percentage of granules occurring in each size range for some potato starches*

Midpoint (microns)	Warba (early harvest)	Late harvest			Midpoint (microns)	Warba (early harvest)	Late harvest		
		Warba	Bliss Triumph	Irish Cobbler			Warba	Bliss Triumph	Irish Cobbler
	Percent	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
0	---	---	---	---	48	6.8	3.0	1.2	2.2
4	---	---	---	---	52	4.8	3.4	1.6	1.0
8	1.0	4.6	6.2	5.0	56	3.0	2.0	.2	1.0
12	3.8	9.0	19.0	7.6	60	2.4	2.2	.6	1.8
16	5.0	12.2	15.8	12.2	64	2.2	.2	.2	.2
20	10.6	16.6	16.8	15.6	68	.6	.6	---	.2
24	10.8	8.8	9.8	17.4	72	.8	---	---	.2
28	13.8	8.4	10.4	12.4					
32	10.2	8.0	9.0	9.8					
36	10.2	6.8	3.6	5.6		*Microns*	*Microns*	*Microns*	*Microns*
40	9.2	8.6	4.4	4.6	Average diameter	33.91	28.28	22.40	26.06
44	4.8	5.6	1.2	3.2					

DISCUSSION

Measurements of the viscosity, the granule size, and granule size distribution, and the gelatinization temperatures show that varietal and environmental factors affect the properties of the starch in some manner. It is recognized, however, that the environmental factors in these experiments were restricted both in extent and kind, and interpretations of subsequent comments should be made subject to these limitations. If conditions should prevail in which other factors would be imposed or the same factors would have greater or lesser importance, it is assumed that the results obtained would no longer be valid.

In earlier paragraphs, stress was placed upon the probable importance of granule structure in the pasting characteristics of starches. As the term "structure" was used, it was intended to include every chemical individual which contributed to the make-up of the granule. Küntzel and Doehner (8) have discussed the part played by granule permeability in pasting behavior. It is to be presumed that the permeability may be partly determined by the nature and amounts of adsorbed substances which, in turn, might well vary because of varietal and environmental differences. If this is true, the nature and amounts of adsorbed materials, as well as the granule size and granule size distribution, would affect the viscosity records and gelatinization temperatures of starches from a given source.

VARIETY COMPARISON, POTATOES

When the starches from three varieties of potatoes were compared, it was found that the starch from Warba exhibited the highest gelatinization temperature, the largest granule size, and formed the most viscous paste which thinned out least during cooking. The Bliss Triumph formed a starch paste that had the lowest gelatinization temperature and the smallest granule size, but the maximum viscosity was slightly higher than that of the Irish Cobbler. From this it may be concluded that the varietal factor makes more difference in the viscosity of a paste formed from starch than the granule size or granule size distribution, and that the viscosity is not always reflected in the gelatinization temperature. This may be accounted for by

assuming differences in permeability or internal physical structure, or both.

Of the varieties tested, the Warba produced the best starch for a very viscous paste with the least thinning out when cooked.

VARIETY COMPARISONS, SWEETPOTATOES

When the viscosity examinations were made of the sweetpotato starches, it was found that the viscosity curves of Red Bermuda and Nancy Hall were quite similar in form to those of the Jersey varieties, but the latter curves started 5° to 6° C. higher. The same difference was noticed in the gelatinization temperatures, but the granule sizes were about the same. A comparison of their viscosity showed that the Little Stem Jersey formed the most viscous paste and the one that thinned out the least, while the Big Stem Jersey formed a paste of the lowest viscosity. It may be concluded, therefore, that the varietal factor of sweetpotatoes will make more difference in the quality of the starch obtained than the granule size or size distribution.

Of the varieties tested, the Little Stem Jersey formed a starch that produced the most viscous paste and the paste that thinned out the least.

EFFECT OF CURING UPON STARCH FROM SWEETPOTATOES

Both the cured and uncured samples were taken from the same lot of sweetpotatoes. It was observed that curing caused similar changes in the properties of the starches obtained from all varieties. Its effect was to lower the gelatinization temperature, give the starch granules a smaller average size, and materially decrease the viscosity of the starch pastes. If it may be accepted that, other things being equal, the gelatinization temperature (*11*) and the viscosity (*14, 6*) are higher the smaller the granule diameter, it follows that curing, by lowering the gelatinization temperature and viscosity with a decrease in granule size, modified the structure of the starch granule.

EFFECT OF TIME OF HARVEST UPON POTATOES

The varieties of potatoes harvested at different periods were the Bliss Triumph, Warba, and Irish Cobbler. It was found that at late harvest, the gelatinization temperature of the starch was higher (except for the Warba, which did not change) and both the viscosity and the temperature of the initial development of viscosity were lower. But the effect of late harvest upon the granule size varied with the variety; granules of the Cobbler became larger, those of Bliss Triumph remained the same, and those of the Warba became smaller at late harvest. It accordingly is apparent that alteration of the behavior of starch due to the time of harvest is not predictable on the basis of changes in granule size. Here, as in the case of sweetpotato cure, there is the suggestion that the granules have undergone structural modification.

COMPARISON OF POTATO AND SWEETPOTATO STARCHES

Native potato and sweetpotato starches differed from each other in almost every respect. The gelatinization temperature of the potato starch was lower, the average granule size was much larger, and the

size distribution of the granules was over a wider range than the sweet-potato starches. But the greatest difference between them was to be found in their viscosities. The general forms of the two viscosity curves were different; sweetpotato starch formed a paste that became quite viscous within a few degrees above the lower limit of the gela-tinization temperature, began to thin out immediately on reaching the upper limit, and when cooled was never highly viscous. Potato starch always began its viscosity curve below the lower limit of the gelatinization temperature, developed its maximum viscosity more slowly, but after it was attained, did not thin out to any great extent. Both starches formed pastes which were clear. From these observa-tions it appears that native potato starch would possess excellent quality for any purpose requiring a paste of high viscosity, whereas sweetpotato starch should serve for purposes which require a thinner paste.

It was observed that the viscosity curves of the potato starches started below the lower limit of the gelatinization temperatures and continued after the upper limit was reached while those for sweet-potato starches always began at the lower limit and began to thin out as soon as the upper limit was reached. From this it may be inferred that potato starches swell a great deal below and above the gelatiniza-tion temperature, while in sweetpotato starches there must be very little, if any, swelling before the granules begin to lose their anisotropy or after anisotropy has disappeared. It follows, therefore, that the gelatinization temperature, based on the loss of anisotropy, does not necessarily correspond to that part of the viscosity curve between zero viscosity and the initial maximum.

COMPARISON OF NATIVE WITH DOMESTIC COMMERCIAL STARCHES

The study of the quality of native potato and sweetpotato starches would not be adequate without a comparison with domestic commer-cial starches. Starches obtained from Stein, Hall & Co. were used for this purpose.

Figure 5 provides a comparison of the viscosity of a Little Stem Jersey starch with the commercial sweetpotato starch; table 2 shows how the commercial starch compares with native starches with respect to gelatinization temperatures and granule size. It is apparent that the commercial starch, except for its appreciably larger granule size, closely resembles that obtained from the Jersey varieties.

Native potato starch, on the other hand, differs markedly from domestic commercial starch except in granule size. The gelatinization temperature of the latter is considerably lower than that of any of the native starches (table 3) and its viscosity record, although much higher during the heating stage, is similar in general form (fig. 6) to that of sweetpotato starch (fig. 5).

It was only after a series of experiments on the effect of cure on the quality of sweetpotato starches[4] that a satisfactory explanation was found for the marked difference between native potato starches and the commercial starch used. In these experiments results were ob-tained which strongly indicate that pasting behavior is partly deter-mined by the extensiveness of adsorption throughout the starch granule and the nature of the materials adsorbed. Küntzel and

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[4] BARHAM, H. N., and WAGONER, J. A. Unpublished data.

Doehner (7), under similar circumstances, had ascribed differences in pasting behavior to impurities which had not been removed, but no supporting experimental evidence was given.

In order to determine whether this marked difference in pasting behavior was due to adsorbed substances, the commercial starch was exhaustively extracted with 85-percent methanol according to the method of Schoch (13). The viscosity records of both the extracted and unextracted commercial starch together with a native starch (late-harvest Warba) are shown in figure 6. These results indicate
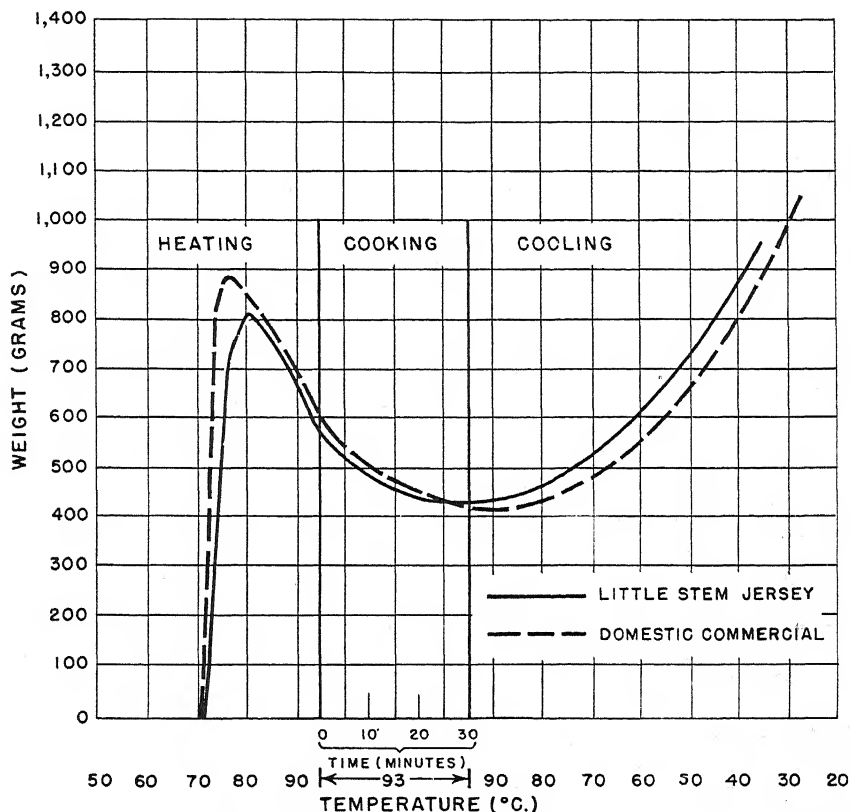
FIGURE 5.—Viscosity curve for native Little Stem Jersey sweetpotato starch compared with that for a domestic commercial sweetpotato starch.

that extraction has had the effect of changing the quality of the extracted starch to the extent that it possesses the same general pasting behavior as that of native starches.

It is not to be concluded, however, that the native starches were of necessity purer. Similar extractions were made also of native potato and of native and commercial sweetpotato starches. Although there were some changes in the viscosity records as a consequence of extraction, the general forms of the curves remained unaltered. Although no conclusions may be drawn from these experiments as to the significance of the amounts of materials adsorbed, the nature of such materials

does appear to have an important bearing on pasting behavior. A discussion of the origin of differences in the nature of the adsorbed materials on potato starches would involve only speculation; there are numerous ways in which such differences could resaonably be accounted for.

CONCLUSIONS

Varietal and environmental differences in potatoes and sweet potatoes were reflected in viscosity, gelatinization temperature, granule size, and granule size frequency measurements of the starches obtained
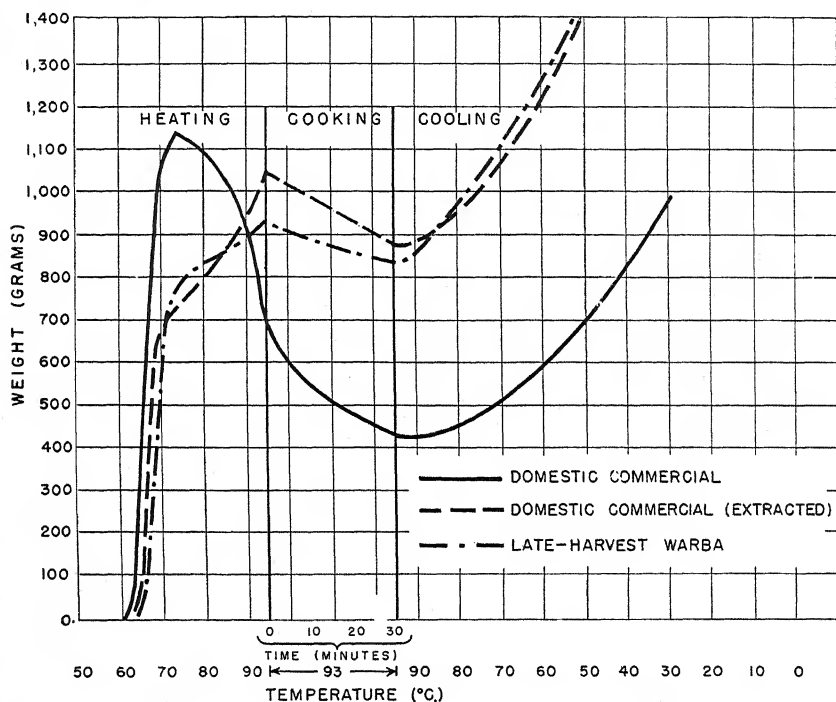
FIGURE 6.—Viscosity curve for native potato starch compared with that for a domestic commercial potato starch.

from them. There was no direct correlation of the granule size and granule size frequencies, as they were affected by such differences, with either the viscosity or the gelatinization temperature, nor were the gelatinization temperature and viscosity directly related. It may be assumed that varietal and environmental factors in some manner affect the structure of the starch granule. That structural differences due to adsorbed materials are capable of markedly affecting the pasting behavior of a starch has been demonstrated.

The gelatinization temperature, based on the loss of anisotropy, does not necessarily correspond to that part of the viscosity curve between zero viscosity and the initial maximum.

Five hundred granules seem to be a sufficient number to count in order to obtain the average size and the size frequency. A smaller number than this does not always permit a reliable statistical treatment.

Curing of the sweetpotatoes lowered the gelatinization temperature, the granule size, and the viscosity of the starches, indicating a change in the nature of the granule during the curing.

The late harvest of potatoes tended to raise the gelatinization temperature and to decrease the viscosity, but the average size of the granules was either smaller or larger, depending upon the variety.

The Warba variety of potatoes and the Little Stem Jersey variety of sweetpotatoes formed the most viscous pastes and the ones that thinned out least during cooking.

Starches obtained from Kansas potatoes and sweetpotatoes are of good quality and are the equal of commercial starches if, in the case of potato starch, the basis for comparison is extracted starch.

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EFFECT OF CORN BARRIERS ON NATURAL CROSSING IN COTTON [1]

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INTRODUCTION

Natural crossing has important implications throughout the cotton industry, from the technique of breeding superior strains to the final utilization of manufactured products. Segregation from uncontrolled hybridization may cause adverse effects in any stage of cotton breeding, production, or utilization in which fiber quality or uniformity is concerned. The quality of cotton lint is dependent on many environmental and genetic factors. Environmental factors, including soil uniformity, fertilization, and cultural practices, are subject to some control; but in the greater part of the Cotton Belt, moisture supply is dependent on rainfall, and since this important factor is not subject to control, the entire complex of ecological factors and responses becomes largely fortuitous. In contrast, the genetic constitution, which determines potential fiber properties, plant characteristics, and yield, may be fixed through careful breeding and protected from contamination in the various stages of multiplication, distribution, and production.

The diversity of plant and fiber characters exhibited by commercial varieties of cotton is due largely to segregation following natural cross-pollination between unlike plants. All types of cotton grown commercially in the United States hybridize readily, and rapid mongrelization occurs when unlike types, varieties, or strains are planted in proximity, unless planting seed from the several lots is protected from natural crossing.

In breeding work with cotton, genetic purity is of major importance in developing superior strains. Breeders may conveniently control pollination in establishing and maintaining purity during the various stages of selection, but when small stocks are increased to multiplication-block or field size, artificial self-pollination becomes too costly, and isolated multiplication of the stocks must be used if mixture is to be prevented. The distance required for adequate isolation and the extent of cross-pollination between close or adjacent plantings of unlike cottons have been studied by numerous investigators. The present study was undertaken to furnish information on the effectiveness of guard rows of corn in protecting cotton multiplication blocks from natural crossing.

LITERATURE REVIEW

Comprehensive reviews of literature on this subject have been given by Kearney (*5, 6*),[2] Brown (*3*), and others. Kearney (*5, p. 11*), in summarizing the results of his own experiments and those of other investigators, states:

* * * The proportion of vicinists rarely exceeds 20 per cent, however, and is usually much smaller. The available information in regard to vicinism therefore points strongly to the conclusion that in cotton self-fertilization greatly predominates over cross-fertilization. It should not be inferred, however, that because most of the ovules normally are self-fertilized, such cross-fertilization as occurs is negligible in its effect upon the uniformity of a variety.

As a rule, the percentage of vicinists decreases rapidly as the distance between the seed-bearing and the pollen-bearing parents increases, but the data at hand do not permit a conclusion to be drawn as to the degree of isolation necessary to eliminate the danger of cross-pollination. This is doubtless affected by the nature of the varieties grown, by local and seasonal variations in the insect population and in the flowering of other plants, and by topography, weather, and other factors.

Ware (*12*) concluded that the amount of natural crossing in cotton varies with the season and locality and is determined by the number of insects present and capable of carrying pollen from one flower to another. He also called attention to the fact that much natural crossing is never detected, since it can be observed only when the pollen is carried from a plant that possesses a heritable detectable difference.

From experiments conducted at State College, Miss., in 1918, Brown (*3*) reported 3.9 to 5.6 percent of crossing on rows of green-leaf cotton growing next to red-leaf cotton, less than 1 percent on the 2d to 8th rows from the red-leaf, and no crossing on rows 9 to 12. In 1919, however, with a larger amount of red-leaf cotton (27 rows) planted at one end of a field of green-leaf, 14.8 percent hybrids were obtained from seed of the row next to the red-leaf, 3.2 percent on the 5th row, 1.9 percent on the 10th, and from 0.6 percent on the 16th to 0.06 percent on the 114th row. There were 119 rows in the field. Brown attributed the larger amount of crossing in 1919 to the larger number of red-leaf plants included in the experiment. Richmond et al. (*9*) reported an average of 9 percent of natural crossing between upland and sea-island stocks from experiments conducted in Texas. Allard (*1*), Balls (*2*), McLendon (*7*), the Mississippi station (*8*), Shoemaker (*10*), Stroman and Mahoney (*11*), Webber (*13*), and others have reported amounts of natural crossing observed ranging from less than 1 to more than 60 percent.

It is evident from the literature that climatic and biotic factors greatly influence the amount of natural crossing. Although serious consideration has been given to the effect of insect populations and distances between plantings of unlike parents, an equal abundance of unlike pollen has been lacking in many previous studies. Equality in amount of pollen would be most nearly approximated with equal areas of unlike plants. Many previous experiments have consisted of single rows or small plots of red-leaf plants entirely surrounded by green-leaf plants. Data have been given showing the extent of crossing in rows of green-leaf cotton adjacent to red-leaf and in rows of green-leaf removed by 5, 10, 20, or more intervening rows from red-leaf cotton. Data of this nature must be interpreted with care, insofar

[2] Italic numbers in parentheses refer to Literature Cited, p. 360.

as application to practical problems of increase is concerned, since the amount of pollen available from normal plants was, in many of the reports cited, considerably greater than that from the contrasting genotype.

METHOD OF INVESTIGATION

In breeding work it usually is necessary to plant numerous increase blocks of different strains for the multiplication of seed from superior progenies. Complete isolation is frequently difficult to arrange, and it has been a common practice to plant multiplication blocks within short distances of each other with or without intervening rows of corn or other tall-growing crops. On the basis of previous information, such practices might be assumed to afford a fair degree of isolation.

Experiments on natural crossing in cotton were conducted at Knoxville, Tenn., in 1939–40 and 1940–41 to test the practicability of corn barriers in protecting unlike cottons from natural crossing. Upland cottons (*Gossypium hirsutum* L.) possessing red-plant and green-plant colors were chosen for the unlike parents, since the intermediate inheritance of plant color (*12*) provides a convenient means of identifying the hybrids. Equal areas of the contrasting types were included in the experiments in order that the study might be representative of practical field requirements and might also provide a simple basis for comparison.

The plan of planting in each season provided four test conditions: (1) Green adjacent to red but guarded from any other cotton by corn barriers; (2) green separated from red by three rows of corn; (3) green separated from red by six rows of corn; and (4) green separated from red by nine rows of corn (fig. 1). In 1939 four-row plots were used, but owing to limited space in 1940 three-row plots were used for both green and red genotypes. The four replications of each test condition were arranged in a Latin square to reduce the effect of external factors, such as the direction of prevailing winds, insect habitats, and adjoining crops.

The plots of red-plant and green-plant cotton and the corn barriers were planted on the same date. Flowering in the two kinds of cotton covered approximately the same period, but the green variety was somewhat more prolific than the red, especially in the 1940 field planting. In both seasons the corn barriers reached a height of 8 to 10 feet and were well advanced in growth before the cotton began to flower.

Seed cotton was harvested and ginned by rows from all plots of green plants, and the seed from each row was planted the following spring in a corresponding 100-foot row in order that the amount of hydridization might be measured by the proportion of intermediate red plants. The total number of plants thus grown was 86,951, from the 1939–40 experiment, and 50,256, from the 1940–41 experiment. Records were made of the total number of plants and hybrids in each row, and from these data the percentage of hybrids was calculated.

In 1941 the scope of the study was extended to cover the effect of isolation distances, ranging from approximately 0.1 to 0.8 mile, on natural crossing. Small blocks of pure red-leaf cotton were grown at six places surrounding the station; the open-pollinated seed produced
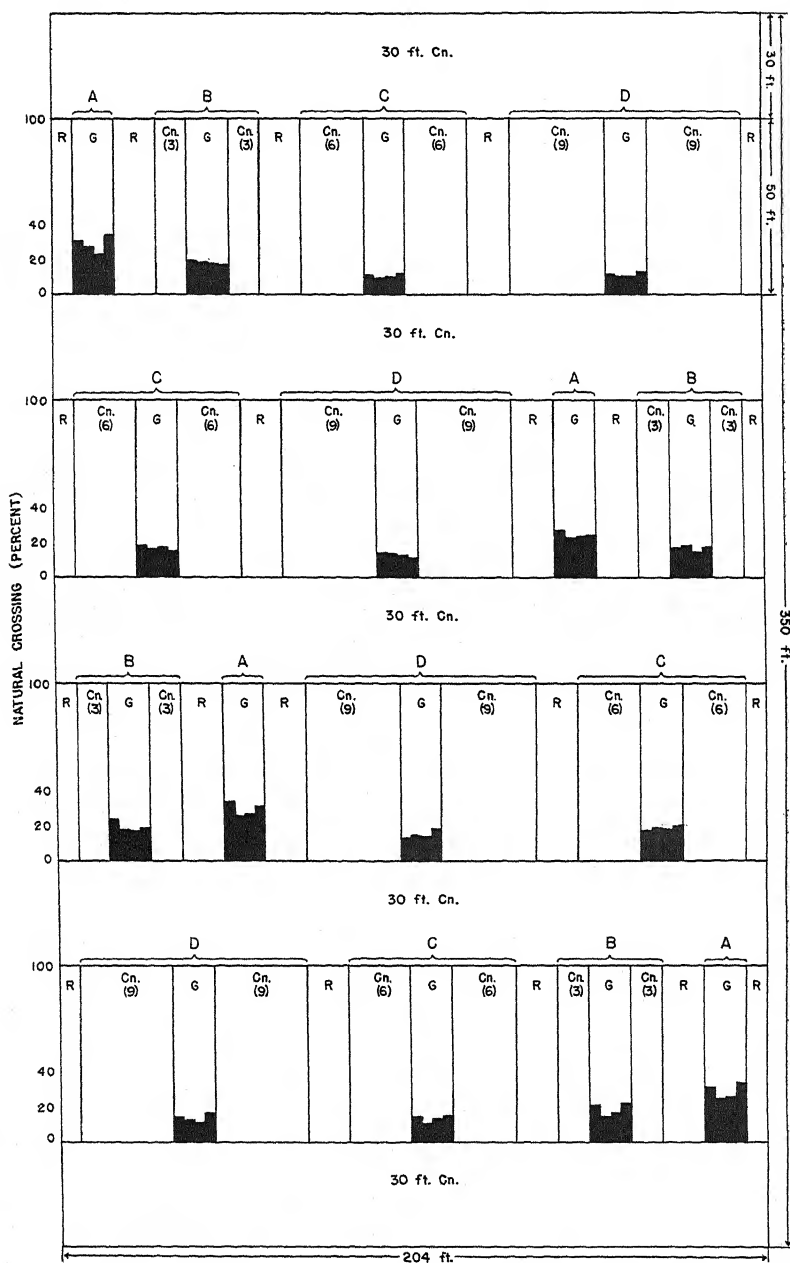
FIGURE 1.—Field plan of natural-crossing experiment in 1939, showing location of green-leaf cotton, red-leaf cotton, and corn barriers. *G* indicates green-leaf cotton, *R* red-leaf cotton, and *Cn.* corn. The numbers in parentheses indicate the number of rows in each corn barrier. The four vertical bars in each green-leaf plot show the percentage of natural hybrids by rows.

in each of the blocks was planted in 1942; and the resulting plants were classified to determine the number of natural hybrids. In this part of the study no artificial barriers were used, so that protection from natural crossing depended on distance, topography, and natural barriers of trees, brush, or intervening fields of other crops or of grassland.

ANALYSIS OF DATA

Data from the 1939–40 study on the total number of plants and the percentage of red plants are shown by plots and rows in table 1. For convenience in comparing the data, the 4 replicates for no barrier, 3-row barrier, 6-row barrier, and 9-row barrier of corn, respectively, are grouped together. The total plant count and the percentage of natural crosses are shown by rows and plots for the reason that in the comprehensive analysis it seemed desirable to compare the percentage of natural hybrids in the outside and inside rows of the various plots. The total number of plants per row, though in all cases adequate for a satisfactory percentage determination, ranged from approximately 1,050 to more than 1,600; consequently, percentages as computed from the plot totals and as averaged for separate rows differed slightly. Under these circumstances, a simple analysis of variance based on percentages calculated from plot totals will not coincide exactly with the whole-plot section in the comprehensive analysis of variance, in

TABLE 1.—*Total number of plants and the percentage of red plants by plots and rows, natural crossing study, Knoxville, Tenn., 1939–40*

No barrier			3-row corn barrier			6-row corn barrier			9-row corn barrier		
Plot and row	Total plants	Intermediate red plants	Plot and row	Total plants	Intermediate red plants	Plot and row	Total plants	Intermediate red plants	Plot and row	Total plants	Intermediate red plants
	Number	*Percent*		*Number*	*Percent*		*Number*	*Percent*		*Number*	*Percent*
I-A-1	1,414	30.2	I-B-1	1,417	19.2	-C-1	1,498	11.1	I-D-1	1,527	11.9
2	1,489	27.1	2	1,353	18.0	2	1,299	9.1	2	1,160	10.8
3	1,566	22.5	3	1,463	17.0	3	1,515	9.4	3	1,274	10.8
4	1,488	33.0	4	1,291	16.7	4	1,425	11.7	4	1,431	12.6
Total	5,957	28.1	Total	5,524	17.7	Total	5,737	10.4	Total	5,392	11.6
II-A-1	1,052	27.2	II-B-1	1,273	17.0	II-C-1	1,356	18.2	II-D-1	1,256	13.4
2	1,446	22.1	2	1,343	18.4	2	1,421	16.0	2	1,507	12.4
3	1,158	23.1	3	1,239	14.9	3	1,342	16.6	3	1,255	12.2
4	1,617	23.5	4	1,450	17.0	4	1,519	15.5	4	1,429	11.8
Total	5,273	23.7	Total	5,305	16.9	Total	5,638	16.5	Total	5,447	12.4
III-A-1	1,268	33.4	III-B-1	1,066	23.7	III-C-1	1,406	16.8	III-D-1	1,139	12.4
2	1,450	25.0	2	1,514	16.4	2	1,197	18.9	2	1,368	13.2
3	1,372	26.0	3	1,460	16.2	3	1,207	17.4	3	1,142	12.7
4	1,406	31.5	4	1,534	18.3	4	1,433	19.8	4	1,479	18.1
Total	5,496	28.8	Total	5,574	18.3	Total	5,243	18.2	Total	5,128	14.3
IV-A-1	1,071	31.1	IV-B-1	1,236	20.3	IV-C-1	1,311	14.8	IV-D-1	1,509	14.6
2	1,421	24.1	2	1,448	15.5	2	1,296	11.2	2	1,273	12.5
3	1,159	24.5	3	1,196	16.6	3	1,476	13.1	3	1,484	11.8
4	1,439	33.0	4	1,354	21.6	4	1,185	15.3	4	1,379	15.1
Total	5,090	28.2	Total	5,234	18.5	Total	5,268	13.5	Total	5,645	13.5
Grand total	21,816	27.2	Grand total	21,637	17.8	Grand total	21,886	14.6	Grand total	21,612	12.9

which the split-plot treatment permits estimating the contributions of outside and inside rows and interactions. Since no substantial differences were found, either in F values or in requirement for significance, between the two bases of calculation, only the comprehensive analysis is shown (table 2).

TABLE 2.—*Analysis of variance of data obtained in the natural crossing study, Knoxville, Tenn., 1939–40*

Variance	Degrees of freedom	Mean square	F		
			Found	Required for—	
				99:1	19:1
Whole plot:					
Barriers, linear	1	1,733.99	116.77	13.74	5.99
Barriers, quadratic	1	232.18	15.64	13.74	5.99
Barriers, cubic	1	17.72	1.19	13.74	5.99
Rows	3	28.72	1.93	9.78	4.76
Columns	3	18.54	1.25	9.78	4.76
Error (a)	6	14.85			
Split plot:					
Outside vs. inside rows	1	138.95	69.47	13.74	5.99
Outside vs. inside rows×barriers, linear	1	40.83	20.41	13.74	5.99
Outside vs. inside rows×barriers, quadratic	1	13.78	6.89	13.74	5.99
Outside vs. inside rows×barriers, cubic	1	.16			
Outside vs. inside rows×rows	3	9.12	4.06	9.78	4.76
Outside vs. inside rows×columns	3	1.22			
Error (b)	6	2.00			
Error (c)	32	2.55			
Total	63	40.21			

As a matter of convenience in interpretation, individual comparisons are tabulated for test conditions and for the interactions of inside vs. outside rows with test conditions (table 2). Variances due to linear, quadratic, and cubic degrees of freedom were calculated from the table of coefficients for orthogonal polynomials of Fisher and Yates (4). A study of mean squares for the whole-plot section of the analysis indicates that certain test conditions were effective in reducing the amount of natural crossing. These individual comparisons are based on all of the treatments, so that the no-barrier comparisons as well as the three degrees of guarding are evaluated in the set of individual degrees of freedom shown in table 2. The linear component is decidedly significant, as is indicated by the F value found (116.77), in comparison with 13.74 required for odds of 99:1. The quadratic component barely exceeds odds of 99:1, but indicates that the relationship departs significantly from linearity. An examination of the data reveals that the decrease from no barrier to three rows of corn is proportionately greater than the decreases obtained with successively greater barriers, and this finding suggests that the following specification of individual comparisons is capable of providing further information:

Variance:	Degrees of freedom	Mean square
Barriers, linear (3 vs. 9 rows of corn)	1	202.51
Barriers, quadratic (3 plus 9 vs. 6 rows of corn)	1	5.65
Barriers vs. no barriers	1	1,775.73

In this set of individual comparisons, the linear and quadratic components are estimated on the guarded conditions and the third individual comparison is between all guarded and no barriers. In this set the linear degree of freedom closely approaches odds of 99:1 and the quadratic is not materially different from error. The high contribution for barriers vs. no barriers indicates clearly the differential amount of natural crossing between the unguarded and guarded conditions. The whole-plot section of the analysis, therefore, shows that the use of corn barriers reduces the amount of natural crossing and that the effect of the number of guard rows tends to linearity.
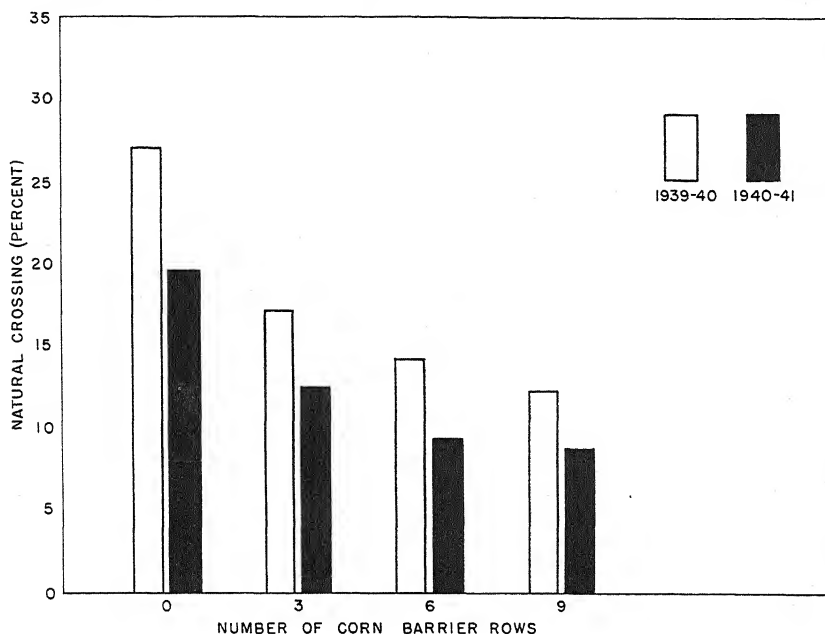
FIGURE 2.—Percentages of natural crossing as averages of four replicates by barrier conditions and years.

Better protection is indicated for plots of cotton surrounded by a larger number of corn rows than for plots surrounded by a smaller number of corn rows, but the means of the various barrier conditions (table 1 and fig. 2) show a proportionately smaller decrease from six- to nine-row than from three- to six-row barriers, and this suggests that an extrapolation of the indicated linearity should not be attempted.

Despite the indicated relative efficiency of different numbers of guard rows, the conclusion must be drawn that the maximum width of corn barriers used in this study was not sufficient to protect pure-line multiplication blocks satisfactorily.

Approximately 10 percent of natural crossing was found with the use of nine-row corn barriers. This amount, or even one-half or one-third as much, in each of the first few generations of multiplication, would largely defeat the objectives of pedigree inbreeding. Better

isolation than was provided by nine rows of corn must be obtained in order to insure a sufficient homozygosity of stocks to permit continued production for several years without the familiar deterioration or "running out" of varieties. The use of corn barriers greatly exceeding nine rows would require a large amount of land if many stocks are being multiplied. Since no evidence is available concerning the minimum number of corn rows that would be required to give adequate protection from natural crossing, it seems more practical to use multiplication blocks isolated by at least one-half mile, and preferably 1 mile, from other cotton varieties. Isolation is difficult to obtain in some sections of the Cotton Belt, but in most cotton States there are localities in which little or no cotton is grown, although climatic and soil conditions are favorable for a fair production. Such localities offer a good opportunity for the economical and safe increase of carefully controlled breeding stocks.

The split-plot section of the analysis (table 2) shows the variance for inside vs. outside rows and interactions for the 1939–40 study. The mean square for outside vs. inside rows is highly significant, and this indicates that, in this study, the number of natural hybrids was greater on the outside rows of the green plots than on the inside rows. Individual degrees of freedom are tabulated for interactions of outside vs. inside rows with test conditions, and these comparisons show that the relative differential between outside and inside rows was associated with the linear degree of freedom for barriers. An examination of the data shows that this interaction was due largely to a greater differential between the no-barrier treatment and treatments consisting of a large number of guard rows. A logical explanation for this differential may be found in the flight habits of the insects largely responsible for natural crossing. Bumblebees, which appear to be the most active agents in natural crossing, frequently move only short distances between successive visits to flowers, and this fact probably accounts for the higher amount of natural crossing on outside rows of the unguarded plots. Conversely, when the distance between plots is rather great, the insects apparently enter the new plot in a substantially random manner, and this results in less distinction between outside and inside rows.

A graphic presentation of the results by rows for the 1939–40 experiment is shown in figure 1. The position of treatments in the various replicates coincides with the field planting plan, so that figure 1 not only presents the actual data by rows but also serves to show the field arrangement of red-leaf cotton, green-leaf cotton, and corn barriers.

The natural crossing experiment of 1940–41 was similar to that of 1939–40 in all details except that three-row plots were used. The results of the two experiments were so closely comparable that a detailed presentation of the data for 1940–41 will be omitted and only summaries shown. The general level of natural crossing was somewhat lower in the second year, the averages ranging from slightly less than 10 to nearly 20 percent, as is evident in the comparison of barrier effects for the 2 years (fig. 2).

The lower level of natural crossing in the second experiment might be due either to the presence of fewer insects or to a proportionately smaller number of flowers on the red plants. Facilities were not available for accurately comparing insect populations in the two

seasons, and consequently no conclusions may be drawn on this point. In each experiment equal plot areas were planted to green-leaf and red-leaf cotton, and the two kinds were thinned in the same manner, so that closely equivalent plant numbers of the contrasting types reached maturity. It was noted, however, that red plants were somewhat less prolific than green plants in the number of flowers produced in 1939 and considerably less prolific than green plants in 1940. These observations suggest that the percentages of natural crossing found are conservative in each of the years when interpreted as amount of natural crossing expected in multiplication blocks where equal prolificacy might be assumed.

An analysis of variance for the 1940–41 data is shown in table 3. It will be noted that this analysis is in general consistent with that for the preceding year's data; consequently it does not seem necessary to discuss the results in detail, except to mention that outside vs. inside rows failed to reach significance in the second year of the study.

These experiments were designed to measure the natural crossing between red and green phenotypes, but it was not possible to detect crossing among the various green plots. It follows that the amounts tabulated for the various conditions actually represent only a part of the natural crossing which occurred between plots. The study in each year consisted of 16-plot units of green, which were used to measure the natural crossing from 16-plot units of red. If these conditions are considered in relation to a practical use of corn barriers where 32 plots of pedigreed inbred cotton stocks were being multiplied, natural crossing from any of the other 31 plots would constitute contamination, and on this basis it might be estimated that the actual amount of crossing among plots was approximately double the amount detected.

These experiments were not designed to measure sib crossing within plots. On the basis of evidence available that amount of natural crossing is inversely related to distance between the parents, it might be expected that sib crossing within plots would exceed that between plots. However, in pure-line increase it may be expected that the

TABLE 3.—*Analysis of variance of data obtained in the natural crossing study, Knoxville, Tenn., 1940–41*

Variance	Degrees of freedom	Mean square	F		
			Found	Required for—	
				99:1	19:1
Whole plot:					
Barriers, linear	1	767.84	111.44	13.74	5.99
Barriers, quadratic	1	148.12	21.50	13.74	5.99
Barriers, cubic	1	1.71			
Rows	3	11.32	1.64	9.78	4.76
Columns	3	41.09	5.96	9.78	4.76
Error (a)	6	6.89			
Split plot:					
Outside vs. inside rows	1	9.86	2.40	13.74	5.99
Outside vs. inside rows × barriers, linear	1	16.88	4.12	13.74	5.99
Outside vs. inside rows × barriers, quadratic	1	1.70			
Outside vs. inside rows × barriers, cubic	1	.05			
Outside vs. inside rows × rows	3	4.75	1.16	9.78	4.76
Outside vs. inside rows × columns	3	.88			
Error (b)	6	4.10			
Error (c)	16	8.17			
Total	47	28.02			

progeny of sib crosses within lines will behave in the same way as the population from self-fertilized seed and that consequently natural crossing within plots would be of no practical consequence in the isolated multiplication of pure lines.

NATURAL CROSSING IN ISOLATED MULTIPLICATION BLOCKS

The need for isolation distances exceeding those practicable with corn barriers, as indicated by results from the 1939–40 study, led to an extension of the experiment to include growing small blocks of pure red-leaf cotton on adjacent farms at known distances from green-leaf cotton at the station. The distance and direction from the station for each of the six blocks of pure red-leaf cotton grown in 1941, and the percentage of natural crossing found for each, are shown in figure 3.
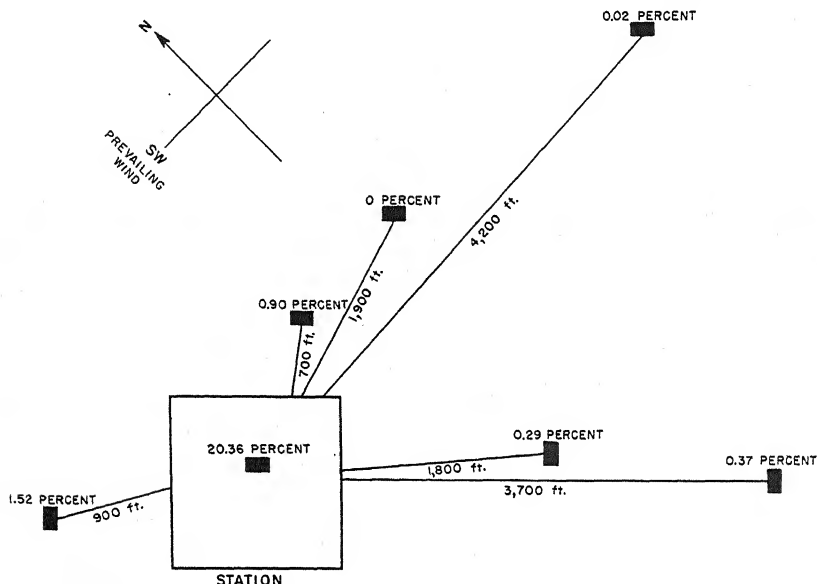
FIGURE 3.—Distance and direction from green-leaf cotton of isolated blocks of red-leaf cotton with percentages of natural crossing.

Seed produced in each of the isolated blocks was grown in 1942, and the plants were classified for color. The total number of plants, the number of hybrid plants, and the percentage of natural crossing are shown in table 4. In addition to the off-station isolated red blocks, a station planting of adjacent red and green rows was included as a check on the general level of natural crossing during the 1941 growing season. A test planting of red selfed seed was grown and classified with the other lots in 1942 as a check on the homozygosity of the red stocks used in planting the 1941 isolated blocks.

It will be noted that the amount of natural crossing is not entirely consistent with the distance of isolation. The 700-foot and 900-foot plantings were separated from the station by open fields of corn and grassland. Small patches of timber grew between the station and the other plantings, and these, together with the rolling topography,

TABLE 4.—*Natural crossing in isolated blocks, Knoxville, Tenn., 1941*

Location of red cotton	Distance from green cotton	Total plants	Hybrid plants	
	Feet (¹)	*Number*	*Number*	*Percent*
Station		3,089	629	20. 36
Isolated block:				
A	900	3,617	55	1. 52
B	700	3,223	29	. 90
C	1,900	5,333	0	0
D	4,200	4,336	1	. 02
E	1,800	3,777	11	. 29
F	3,700	3,528	13	. 37
Check (selfed)		3,978	0	0

¹ Station planting of adjacent red and green rows.

undoubtedly introduced certain inequalities in the relation of distance to amount of natural crossing.

These data indicate that the cotton breeder should decide on the amount of natural crossing that may be tolerated in a particular program of multiplication and distribution, and choose isolation distances accordingly. The genetic homozygosity of the parental stocks to be increased is a primary consideration in deciding on the amount of natural crossing that may be tolerated. If stocks have been inbred 5 years or more and seem promising enough to warrant long-time production, it seems logical that each stage of multiplication should be isolated sufficiently to maintain the genetic purity.

NOTES AND OBSERVATIONS ON INSECTS

It was not possible to make extensive studies on insect populations, but a collection was made during a few short periods in the field. No attempt was made to capture all the insects on the cotton flowers, but rather to get one or a few of each of the species observed. A list of the insects found on cotton flowers follows.[3]

Insects most probably concerned in transfer of pollen:

                                                      Common name

    Bombus spp. (Bombidae) _____ Bumblebee.
    Melissodes obliqua Say? (Anthophoridae) ____ Soil-nesting bee.
    Melissodes spp. (Anthophoridae) _____ Do.
    Halictus ligatus Say (Halictidae) _____ Do.
    Halictus (Chloralictus) spp. (Halictidae) _____ Do.
    Augochlorella spp. (Halictidae) _____ Do.
    Coleomegilla maculata (Degeer) (Coccinellidae)__ Ladybeetle.
    Diabrotica duodecimpunctata (F.) (Chryso- Spotted cucumber beetle.
      melidae)
    Epicauta trichrus (Pallas) (Meloidae) _____ Blister beetle.

Insects less probably concerned in transfer of pollen:

    Formica pallidefulva schaufussi Mayr (For- Pale-yellow ant.
      micidae)
    Dexiini, genus and species uncertain (Tachin- Tachinid fly.
      idae)
    Olla oculata (Say) (Coccinellidae) _____ Ladybeetle.
    Cycloneda munda (Say) (Coccinellidae) _____ Do.
    Hippodamia convergens Guér. (Coccinellidae)__ Convergent ladybeetle.
    Centrinaspis sp. (Curculionidae) _____ Weevil.
    Acrosternum hilare (Say) _____ Green stinkbug.

[3] Identification made through courtesy of Dr. C. F. W. Muesebeck, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture.

Insects less probably concerned in transfer of pollen—Continued:

Podisus maculiventris (Say) _____ Spined soldier bug.
Geocoris punctipes (Say) _____ Big-eyed bug.
Lygus oblineatus (Say) _____ Tarnished plant bug.
Gypona sp., nymph (Homoptera, Cicadel- Leafhopper.
  lidae).
Diabrotica vittata (F.) (Chrysomelidae) _____ Striped cucumber beetle.
Colaspis sp., near *brunnea* (F.) (Chryso- Leaf beetle.
  melidae).
Collops quadrimaculatus (F.) (Malachiidae) ___ Malachiid beetle.
Lebia sp. (Carabidae) _____ Ground beetle.
Psammocharidae (not further determinable)___ Spider wasp.

The separation of the insects into groups more and less probably concerned in natural crossing was made on the basis of feeding habits and other characteristics. Field observations indicate that bumblebees were the most numerous and active insects visiting cotton flowers. Honeybees were observed but not captured, and therefore are not included in the foregoing list. Other insects were present, but the various species of bees were the only insects that were observed to make rapid and rather systematic visits to the flowers. Movement of the bees within the plot usually was only a few feet at a time and from plant to plant along the row. Flight from the plot was usually through the corn barriers rather than up and over the top of the corn.

PRODUCTION OF HYBRID SEED FOR COMMERCIAL PLANTING

At various times the question has been raised whether a practical and inexpensive technique could be developed to produce hybrid cotton seed for commercial plantings. Artificial crossing is too expensive to be profitable on the basis of increases in yield usually expected for hybrid over other stocks within a species. However, in some species crosses, such as sea island × upland (*Gossypium barbadense* × *G. hirsutum*), certain combinations of lines usually give a marked increase in yield over the sea-island parent together with fiber length similar to sea island. These increases depend to a considerable degree on the specific lines used in crossing; and more work in identifying the best lines, studying the technique of producing crosses, and solving certain ginning problems is needed before hybrid production may be recommended.

In the present study the area of land in green- and red-leaf cotton was the same, and the stand was substantially equal, but flower production in the green plots was materially better than in the red. It follows that, despite care in designing the experiment, absolute equality in opportunity was not achieved, and consequently the 20 to 25 percent of natural crossing found in the no-barrier plots is undoubtedly conservative for the conditions under which the tests were made.

Maximum cross-pollination would be expected in areas having large insect populations and in fields near pastures, meadows, or wasteland. In the commercial production of natural crosses it would be logical to plant alternate rows or alternate hills of the contrasting parental stocks, since such an arrangement might be expected to give a larger percentage of hybrids than would occur in adjacent block plantings, such as were used in this study. Seed produced by both parents could be used for planting, if the hybrids are clearly distinguishable in the seedling stage. Any contrasting pair of characters exhibiting inter-

mediate dominance of the 1:2:1 ratio type may be used if the intermediate class is easily distinguishable in the seedling stage. Hand thinning would be required, and the characters used should exhibit marked contrast, so that field workers could be trained to easily recognize and accurately remove the homozygous types.

If the amount of seed planted, including natural crosses, is five to eight times the desired number of plants in the final stand, it might be expected that the thinning-out of all plants, except intermediate ones, would give a satisfactory stand. With hill-dropping 18 to 24
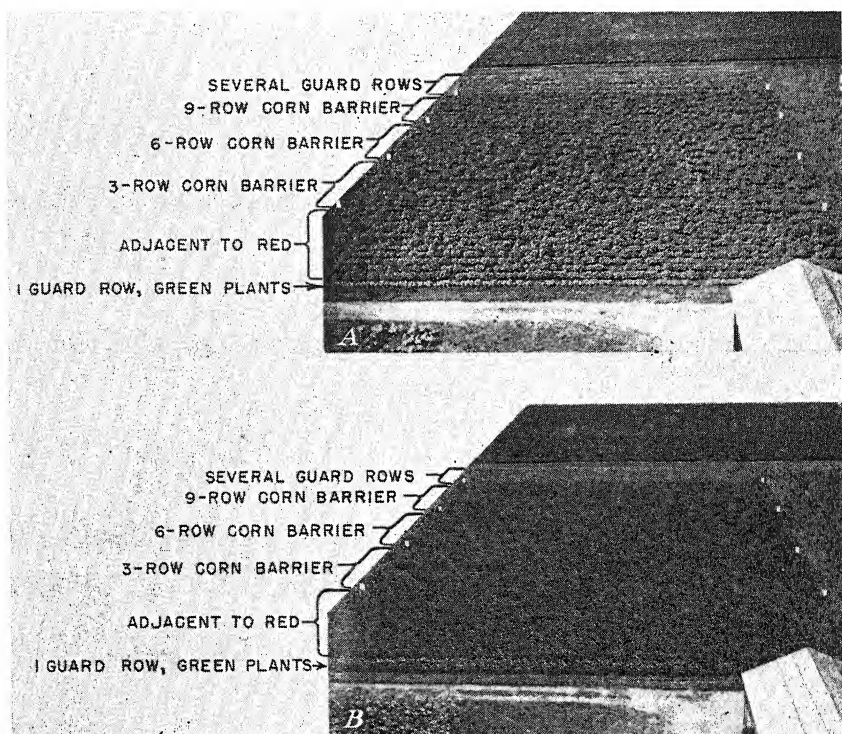
FIGURE 4.—Field views of natural crosses, showing the proportionate stand from the various conditions of guarding: *A*, June 27; *B*, August 22.

inches apart and hand thinning, a hybrid crop might be planted with approximately the same amount of seed ordinarily used when conventional planting and chopping practices are followed.

Figure 4 illustrates the feasibility of obtaining stands when using natural-cross hybrids. Both field views are of the 1941 testing area, showing the appearance of the block on June 27 (*A*) and on August 22 (*B*). The rate of planting of each lot was approximately 1½ bushels per acre. The plants in the area bounded by white stakes are all intermediate red with the exception of one border row next to the roadway in the foreground. This block was used in 1941 for planting the 1940 seed produced on green plots, and, in thinning, all green plants were removed and the intermediate red plants were permitted to remain. The sections are arranged in order of barrier treatment. The first 12

rows from the foreground border to the first pair of white stakes are from the adjacent red-green plots, and it will be noted that the stand is reasonably satisfactory. The successive blocks of 12 rows, in the direction of the background, are from the plots protected by the three-six-, and nine-row corn barriers, respectively, and in these sections the stand decreases progressively in proportion to the amount of natural crossing. Figure 4 shows the results for the year in which the rate of natural crossing was lowest, but illustrates the conclusion that the production of naturally crossed seed, for use in commercial plantings, appears feasible.

SUMMARY AND CONCLUSIONS

A study was conducted for 2 years on the effectiveness of guard plantings of corn on natural crossing in cotton, in which four conditions of guarding were included: (1) Green-leaf adjacent to red-leaf cotton surrounded by corn; (2) green-leaf separated from red-leaf by three rows of corn; (3) green separated from red by six rows of corn; and (4) green separated from red by nine rows of corn.

The amount of natural crossing was determined by planting approximately 1,500 seeds from each row of the plots of green-leaf cotton and determining the percentage of natural hybrids. The data may be interpreted simply and directly, since equal areas were planted to green- and to red-leaf cotton.

The results indicate that corn barriers are effective in reducing the amount of natural crossing and that the reduction tends toward linearity for the different barrier widths used in this experiment.

Despite the indicated efficiency of barriers in reducing the amount of natural crossing, the results show clearly that the barrier widths of corn used did not afford sufficient protection, under the conditions of this experiment, for the multiplication of selfed-line seed stocks. The minimum amount of natural crossing found would in a few generations of multiplication reduce the homozygosity to a point where the seed stocks would be too badly mixed for continued production.

Small-block plantings of red-leaf cotton, made at distances ranging from 700 to 4,200 feet from green-leaf cotton, established the occurrence of natural crossing at distances up to 0.8 mile. It therefore seems clear that distances of 1 mile or more will be required to provide complete isolation, under the conditions prevailing in this study.

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STEM-END ROT OF ORANGES AND FACTORS AFFECTING ITS CONTROL [1]

By Charles Brooks [2]

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INTRODUCTION

Stem-end rot of citrus fruit has received a great deal of critical study and has been the subject of many scientific and popular publications. In spite of the excellent work pertinent to its control, however, it remains a major cause of losses to the retailer and consumer as well as to the grower and shipper of citrus fruit from the southeastern part of the United States. In a study of storage diseases of grapefruit a few years ago, Brooks and McColloch (4)[3] found that 12 to 60 percent (average 30 percent) of the Florida fruit purchased on the market in Washington, D. C., developed stem-end rot if held in storage at 50° F. or higher for as long as 8 weeks.

In the present studies stem-end rot was found to be a serious cause of spoilage of Florida oranges purchased on the Washington market. When the fruit was held for a week at a temperature of 75° F. in an atmosphere having a high relative humidity, stem-end rot resulting from infection in the grove occurred on 7 to 47 percent (average 28 percent) and was the main cause of loss. It seems evident that this disease is the most important cause of loss in Florida oranges to the retailer and the consumer. Much of this loss may be due to failure to use such methods of control as are available, but comprehensive investigations by Winston (13) indicate that part of the trouble may be due to a lack of methods that are completely satisfactory.

As most of the previous studies of stem-end rot have been made on fruit infected in the grove, it was thought desirable in preliminary experiments to inoculate oranges that were known to be free from stem-end rot and then to disinfect them. The results of such experiments as well as of those on naturally infected oranges are reported herein.

MATERIAL AND METHODS

The earlier investigations were conducted at Beltsville, Md., on oranges purchased on the Washington market. The fruits were as fresh as could be found, but in many cases the buttons had lost considerable of their greenness and freshness. In most cases, California oranges were used since these were free from stem-end rot, but the results obtained were checked with Florida oranges both for disease control and for possible injury to the fruit. The later experiments,

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1940–42, were made at Orlando, Fla., on naturally inoculated fruit fresh from the tree.

Under each experimental condition at Beltsville, 18 or more California oranges were used, and the number of Florida fruits was 40 or more, often 100. In the experiments at Orlando 100 or more fruits were used under each condition.

In the inoculation experiments suspensions of *Diplodia* or *Phomopsis* spores were applied to the buttons with a medicine dropper, and the oranges were allowed to stand with the buttons up till the inoculum was nearly dry. Aside from the exceptions mentioned later (pp. 344 and 345), an effort was made to apply the inoculum to both the cut stem and the surrounding calyx. After inoculation the oranges were placed in a large metal cabinet where the temperature was held at about 75° F. and the relative humidity was near the saturation point. In the earlier experiments the fruit was kept in the cabinet throughout the test, but in the later ones it was moved at the end of 7 days to a room where the temperature was 70° and the relative humidity 85 to 90 percent.

In the experiments at Orlando, the oranges were taken as they came from the grove without artificial inoculation. They were carefully sorted, and after disinfection and other treatments were held in a basement room at a temperature of about 70° F. and a relative humidity of 80 to 90 percent.

In testing the effect of gases 5-gallon jars were used and a high humidity was maintained by the use of wet paper towels. When the jars were to remain filled with fruit for a considerable period, small openings were made near the bottom to prevent the accumulation of carbon dioxide. When high-humidity treatment was desired before disinfection, either 5-gallon jars or pony refrigerators were used.

OBTAINING AND GERMINATING SPORES OF THE CAUSAL ORGANISMS

Rapid decay of oranges starting at the buttons or stems may be caused by either of two fungi, *Diplodia natalensis* Pole-Evans [4] or *Phomopsis citri* Fawc. [5] In the course of the experiments a slow stem-end decay caused by a species of *Alternaria* was occasionally found on California fruit. A slow stem-end decay due to *Colletotrichum gloeosporioides* Penz. sometimes occurred on relatively green Florida oranges, especially those previously treated with borax. The present studies were confined largely to the first two organisms.

One of the great difficulties in laboratory studies with these two fungi is that they do not sporulate readily and consistently in culture. In efforts to obtain ready sporulation various types of culture media were tested. The best results with *Phomopsis* were obtained with sterilized snap beans in test tubes. Pods in which the beans had developed to considerable size seemed to be better than those that were less mature. Surplus water was usually drained from the test tubes at the time of inoculation.

Good spore production by *Diplodia* was obtained on seeds of wheat. The wheat was soaked overnight, the excess water was drained off, and the wheat was then sterilized in test tubes or as a shallow layer in

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[4] Perfect stage, *Physalospora rhodina* (Cke.) Berk. and Cke.
[5] The perfect stage, *Diaporthe citri* Wolf, has been reduced by Wehmeyer (*12*) to synonymy with *D. medusaea* Nitschke.

small flasks.   At 80° F. abundant production of hyaline single-celled spores was obtained in 12 to 20 days after inoculation.   If used within 10 days, these spores produced rapid decay of oranges when applied to the buttons in a spore suspension.   When the test-tube or flask cultures were held for much more than 5 weeks at a temperature of 80°, the spores failed to germinate in water, nutrient solutions, or nutrient agar.   This was probably due to some sort of staling or aging, but dryness may also have been a factor since spores were found to be very sensitive to drying after they had issued from the pycnidia. Spores held moist for 15 minutes on a slide and then allowed to dry rapidly failed to germinate later.

Very few brown thick-walled two-celled spores were found in the inoculum obtained from the wheat cultures even several weeks after the spores had issued from the pycnidia.   In some cases actively fruiting cultures were dug out of the flasks or test tubes, broken into small pieces, and allowed to dry slowly in closed Petri plates in the laboratory.   In these, two-celled spores soon predominated and the remaining one-celled spores gave a very low percentage of germination.   When filtered out of a spore suspension and allowed to dry on filter paper, the two-celled spores remained viable for several days under laboratory conditions.

The fact that the spores produced as described did not always prove viable led to germination studies with the different spore forms. These were carried out by placing the spores in water and in prune juice in the concavities of culture slides and exposing them to various storage conditions.   In water the one-celled and the lighter colored two-celled *Diplodia* spores germinated freely, but the darker two-celled *Diplodia* spores and the *Phomopsis* spores often germinated very poorly.   In prune juice, however, all spore forms germinated well.

Since, as discussed on page 373, ethylene treatments are known to increase stem-end rot, tests were made to determine whether any of this increase might be due to better spore germination.   The results showed that exposure in an atmosphere containing 1 to 4,000 ethylene brought the germination of *Phomopsis* and two-celled *Diplodia* spores in water up to more than half that found in prune juice.   Equally great stimulation, however, was obtained by placing two oranges or the rind of two oranges in a 5-gallon jar with the spore cultures. Evidence has been obtained recently (*10*) that oranges give off ethylene.   It is possible that the stimulus from the presence of oranges was due to ethylene, although it is also possible that other gases given off by the oranges may have had some effect.

Vapors from various chemicals were also found to affect germination.   Placing 2 cc. of ethyl acetate, 2 cc. of ethylene chlorhydrin, or a small quantity of menthol in a 5-gallon jar with the water cultures gave a decided increase in germination of two-celled *Diplodia* spores. On the other hand, the vapors given off by geraniol, clove oil, and alcohol had an inhibiting effect.   Stimulation and inhibition of spore germination by volatile substances have been reported by others (*1, 5*).

THE ROLE OF THE STEM BUTTONS

The stem buttons furnish the point of entry for the stem-end rot fungi (fig. 1) and also afford a protected place for the lodgment of spores.

The calyx lobes lie rather close on the surface of the rind, forming a projecting ledge under which spores and dirt can collect and be fairly well protected from wind and sun. Even after several hours of expo-
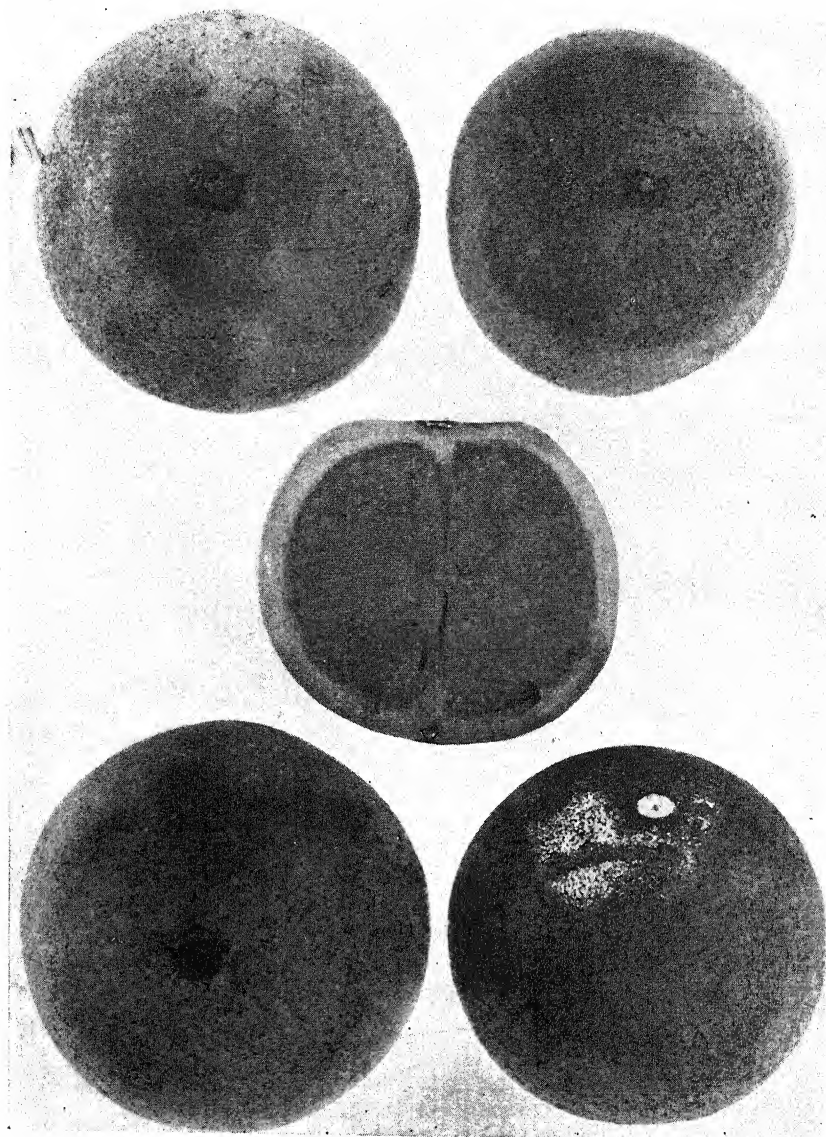
FIGURE 1.—Stem-end rot of oranges at various stages. Note the growth of the causal fungus on the exterior of the orange at lower right.

sure to bright sunshine the fruit surface beneath the calyx does not appear entirely dry when examined with a hand lens.

Stem-end rot can be almost entirely prevented by the early removal of the buttons, but no generally practicable method has been found

for doing this. Winston (*15*) recently showed that in grapefruit grown in certain sections of Florida losses from stem-end rot can be prevented by pulling the fruit and leaving the buttons behind instead of clipping as has been the standard practice. Clipping, however, is necessary for the orange crop because pulling may result in tears in the rind that furnish points of entrance for green and blue molds (*Penicillium digitatum* Sacc. and *P. italicum* Wehmer).

Although it is generally recognized that the stem-end rot fungi gain entrance to the fruits through the buttons, there has been some question whether they enter through the cut stems or through the calyx lobes. Tests were made to obtain evidence on this point.

In one set of experiments a mixture of beeswax, paraffin, and mineral oil was used in paste form to localize the infection. On part of the oranges the cut stems were coated and on others the entire buttons except the cut stems were coated before inoculating with *Phomopsis*. California-grown Washington Navels and Valencias were used in the tests. After 2 weeks at 75° F. 80 percent of the stem-inoculated Washington Navels and 69 percent of the calyx-inoculated ones showed decay; at that time 38 percent of the stem-inoculated Valencias and 13 percent of the calyx-inoculated ones showed decay. When the Valencias were held for an additional 2 weeks, the stem-inoculated fruit showing decay had increased to 92 percent and the calyx-inoculated to 35 percent.

Several tests were made to determine whether decay could be produced by applying spores to the scars left upon the removal of the buttons. The results were contradictory, the degree of infection probably varying inversely with the extent to which separation layers had formed beneath the buttons.

In May 1940 a test was made at Orlando to determine the path of natural infection in freshly harvested Valencia oranges. Merthiolate (sodium ethylmercuri-thiosalicylate) 1 to 1,000 in 50 percent alcohol was applied in one lot to the cut stems only and in another lot to the calyxes only. Part of the fruit was then given a 2-day ethylene treatment, and part was stored immediately. After 4 weeks at 70° F. 45 percent of the ethylene-treated fruit without merthiolate, 19 percent of that with merthiolate applied to the cut stems, and 3 percent of that with merthiolate applied to the calyxes had decay. In similar lots that were not treated with ethylene the percentages were 8, 1, and 0, respectively. In both cases the application to the calyx was more effective than that to the cut stem. However, much more merthiolate was required to cover the calyx than to cover the cut stem, and this quantitative difference may have been significant. In the ethylene-treated fruit most of the decay was due to *Diplodia*, and in the untreated fruit most of it was due to *Phomopsis*.

The results of the various experiments indicate that the stem-end rot fungi can make their start on either the stem or the calyx lobes and probably on any part of the button. These results are in agreement with data reported by Fawcett (*6*).

RELATIVE SPEED OF PENETRATION BY PHOMOPSIS AND DIPLODIA

Evidence as to the relative speed of penetration by the two organisms was obtained by the removal of the buttons or by the application

of a disinfectant at various periods after inoculation and also from records as to the time of appearance of decay on the control lots of fruit. In these experiments the inoculum was applied to all parts of the button. A brief statement of the results has already been published (2).

The effect of the time of removal of the buttons from Washington Navel oranges is shown in table 1, from which it is evident that *Diplodia* makes its way through the button in less than half the time it takes *Phomopsis*.

TABLE 1.—*Effect of time of removal of buttons from inoculated Washington Navel oranges upon the development of stem-end rot*

[*Phomopsis* data, from a single experiment; *Diplodia* data, average of 3 separate experiments]

Organism used	Period between inoculation and button removal	Fruit with stem-end rot—		
		1 week after in-oculation	2 weeks after in-oculation	4 weeks after in-oculation
		Percent	*Percent*	*Percent*
Phomopsis	Check (not removed)	0	68	95
	3 days	0	0	0
	4 days	0	0	4
	6 days	0	5	17
Diplodia	Check (not removed)	79	97	
	1 day	7	12	
	2 days	8	15	
	3 days	43	52	

The effect of the time of borax application upon the development of stem-end rot is shown in table 2. It will be noted that treatment 4 days after inoculation was almost as effective against *Phomopsis* as treatment on the day of inoculation, but that treatments delayed 2 or 3 days were of little value against *Diplodia*. Winston (*14*) pointed out the importance of early disinfection in any attempt to control stem-end rot.

TABLE 2.—*Effect of the time of borax application on the development of stem-end rot in Washington Navel oranges*

Treatments with an 8-percent solution at 100° F. for 2 minutes; fruit held at 75°; A, average of 6 tests; B, average of 5 tests; C, average of 4 tests]

Lot and organism used	Time of borax application	Fruit with stem-end rot—		
		1 week after in-oculation	2 weeks after in-oculation	4 weeks after in-oculation
		Percent	*Percent*	*Percent*
A (*Phomopsis*)	Check (untreated)	0	39	79
	Day of inoculation	0	9	14
	1 day after inoculation	0	3	21
	2 days after inoculation	0	4	15
	3 days after inoculation	0	7	16
	4 days after inoculation	0	11	24
B (*Diplodia*)	Check (untreated)	78	92	
	Day of inoculation	9	30	
	1 day after inoculation	21	33	
C (*Diplodia*)	Check (untreated)	71	98	
	1 day after inoculation	10	44	
	2 days after inoculation	55	84	
	3 days after inoculation	72	93	

It should be noted that in the check lots (table 2) *Diplodia* had produced more decay at the end of 1 and 2 weeks than *Phomopsis* at the end of 2 and 4 weeks, respectively. This contrast in the activity of the two organisms is further emphasized in the experiments reported in figure 2, where it is shown that *Diplodia* had caused as much wastage at the end of 4 and 7 days as *Phomopsis* at the end of 9 and 13 days, respectively. That this difference was not due to temperature is indicated by the fact that the tests were made at approximately the
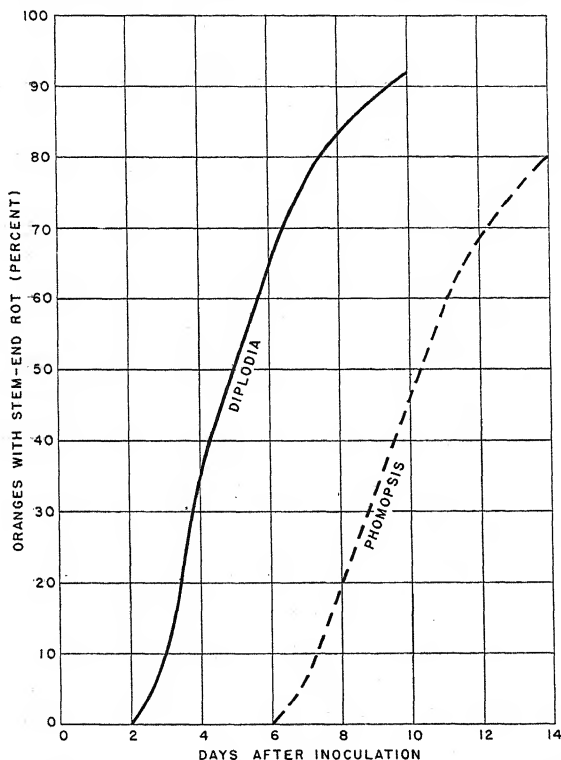
FIGURE 2.—Average rate of development of stem-end rot in Washington Navel oranges inoculated with *Diplodia* and *Phomopsis* and held at approximately 75° F. (Seven tests with each organism.)

optimum for *Phomopsis*, which is 65° to 75° F. (7), and below the optimum (86°) for *Diplodia*.

The various experiments indicate that under conditions favorable to both fungi *Phomopsis* would be so completely outdistanced by *Diplodia* that it would produce little decay.

In one set of inoculation experiments with *Diplodia* a comparison was made between inoculum in which about 99 percent of the spores were one-celled and inoculum in which about 90 percent of the spores were two-celled. Under conditions favorable for the germination of both spore forms (p. 365) there was no difference in the rate at which decay developed.

DISINFECTION EXPERIMENTS WITH HAND-INOCULATED FRUITS

When the present studies were begun it was thought that if fruit known to be free from stem-end rot was used and uniform methods of inoculation were employed the results with each disinfectant would be consistent. But such was not the case. A disinfectant that gave almost complete control in one instance sometimes failed almost entirely in another, and one that stood at the top of the list in one test sometimes fell to the bottom of the list in the next. It was not found advisable, therefore, to accept results without several repetitions.

No completely satisfactory explanation of the variability in results has been found. The degree to which the inoculum was allowed to dry before the fruit was stored and the period between the time of inoculation and the time of disinfection apparently had an important bearing on the results. Evidence was also obtained that a chemical which kills or weakens the buttons without killing all the spores may actually increase decay even though it is known to be a fairly good disinfectant. Since in previous studies (*13, 14*) borax was found to be the best disinfectant for stem-end rot, it was used in all the present experiments for comparison with other materials tested.

FORMALDEHYDE TREATMENTS

A number of experiments were carried out with formaldehyde. Preliminary tests indicated that a solution containing 0.2 percent of formaldehyde was as strong as could be used without injury to the fruit, unless the treatment was to be followed immediately by washing, and that a solution containing 1.5 percent was as strong as could be used even when followed by washing. Even at these strengths formaldehyde sometimes caused a few red specks on some of the oranges.

TABLE 3.—*Stem-end rot of oranges treated with borax and formaldehyde 1 day after inoculation*

[A, Average of 3 tests on Washington Navel and of 1 on Valencia oranges grown in California; B and C, average of 2 and 3 tests, respectively, on Washington Navel oranges grown in California]

Lot and organism used	Treatment	Fruit with stem-end rot —		
		1 week after inoculation	2 weeks after inoculation	4 weeks after inoculation
		Percent	*Percent*	*Percent*
A (*Phomopsis*)	Check (untreated)		93	97
	8 percent borax at 110° F. for 2 minutes; not washed.		27	38
	0.2 percent formaldehyde at 110° F. for 2 minutes; not washed.		15	25
	4 percent borax + 0.2 percent formaldehyde at 100° F. for 2 minutes; not washed.		11	23
B (*Diplodia*)	Check (untreated)	44	64	
	5 percent borax at 75° F. for 2 minutes; not washed.	10	13	
	0.2 percent formaldehyde at 75° F. for 2 minutes; not washed.	42	56	
C (*Diplodia*)	Check (untreated)	79	98	
	5 percent borax at 75° F. for 2 minutes; washed after 2 days.	25	31	
	1.5 percent formaldehyde at 75° F. for 2 minutes; washed immediately.	55	68	

The results of experiments with formaldehyde indicate that it might be of value in the control of decay by *Phomopsis*, but that it is of little or no value in the control of decay by *Diplodia* (table 3). It,

therefore, could not be expected to give satisfactory results with fruit that is likely to carry both fungi. Since there seemed to be no justification for the commercial use of the material, tests were not made to determine whether formaldehyde could be detected in the treated fruit.

CHLORINE TREATMENTS

Calcium and sodium hypochlorites were tested as disinfectants for stem-end rot of oranges. The results are shown in table 4. The treatments as carried out did not cause appreciable injury, but from the standpoint of stem-end rot control they must be regarded as almost complete failures.

TABLE 4.—*Stem-end rot of oranges treated with borax and with chlorine-containing compounds at 80° F. for 2 minutes 1 day after inoculation*

[A, 1 test on Washington Navel oranges; B, average of 3 tests on Washington Navel oranges; C, average of 1 test each on Washington Navel and Florida Pineapple oranges; D, test on Washington Navel oranges; E, average of 3 tests on Washington Navel oranges; F, average of 2 tests on Washington Navel oranges and one on Florida Pineapple oranges; G, average of 2 tests on Washington Navel oranges; Washington Naval oranges grown in California]

Lot and organism used	Treatment	Fruit with stem-end rot—		
		1 week after inoculation	2 weeks after inoculation	4 weeks after inoculation
		Percent	*Percent*	*Percent*
A (*Phomopsis*)	Check (untreated)		58	100
	5 percent borax; not washed		0	0
	Calcium hypochlorite (available chlorine 0.25 percent); not washed		24	76
B (*Diplodia*)	Check (untreated)	30	54	
	5 percent borax; not washed	7	12	
	Calcium hypochlorite (available chlorine 0.25 percent; not washed	15	70	
C (*Diplodia*)	Check (untreated)	54	96	
	5 percent borax; washed after 3 days	26	46	
	Calcium hypochlorite (available chlorine 1.25 percent); washed immediately	59	95	
D (*Phomopsis*)	Check (untreated)		59	100
	5 percent borax; not washed		0	0
	Sodium hypochlorite (available chlorine 0.07 percent); not washed		21	74
E (*Diplodia*)	Check (untreated)	3	31	
	5 percent borax; not washed	0	0	
	Sodium hypochlorite (available chlorine 0.07 percent); not washed	2	20	
F (*Diplodia*)	Check (untreated)	71	83	
	5 percent borax; washed after 1 day	20	22	
	Sodium hypochlorite (available chlorine 0.14 percent); washed immediately	61	81	
G (*Diplodia*)	Check (untreated)	66	85	
	5 percent borax; washed after 2 days	14	33	
	Sodium hypochlorite (available chlorine 0.35 percent); washed immediately	47	64	

PHENATE TREATMENTS

In preliminary experiments two different phenate compounds were tested as orange disinfectants: Sodium ortho-phenylphenate and a trade product that is a mixture of sodium 2-chlorphenylphenate and sodium tetrachlorphenate. In the same concentration the two chemicals gave approximately the same control of stem-end rot. The mixed product was sometimes slightly more effective, but it was much more likely to cause specking and spotting of the oranges and it also had an undesirable effect upon flavor. On some oranges this could not be detected, but when high-quality fruit was used a definite

loss of character and flavor was evident a few days after treatment. No such unfavorable effect was observed with the sodium ortho-phenylphenate.

Van der Plank and Rattray (*11*) reported favorable results in the control of *Penicillium digitatum* (green mold) decay of citrus fruit with a dip in a 0.3- to 0.5-percent solution of sodium ortho-phenyl-phenate. Hwang and Klotz (*9*) obtained decided reduction in the spore germination of *P. digitatum* with a 5-minute exposure to a 0.15-percent solution of the same material.

Sodium ortho-phenylphenate is the only phenyl compound used in the experiments reported, and for the sake of brevity the material is sometimes referred to herein as phenate solution. In preliminary experiments it was not found advisable to use the phenate treatment without following it with washing. When not followed by washing, a 0.2-percent solution left spots on the oranges, yet it did not give satisfactory control of stem-end rot. All the treatments reported in table 5 were followed by washing, and none of them caused injury.

TABLE 5.—*Stem-end rot of oranges treated with borax and phenate at 80° F. for 2 or 3 minutes at different periods after inoculation with Diplodia and held the first week at 75° and the second at 70°*

[A, Florida Pineapple oranges; B to D, California Washington Navel oranges. Fruit in D exposed to ethylene 1 day before disinfecting]

Lot	Treatment	Fruit with stem-end rot—	
		1 week after in-oculation	2 weeks after in-oculation
		Percent	*Percent*
A	Check (untreated)	54	92
	5 percent borax 1 day after inoculation; washed after 3 days	45	67
	1.2 percent phenate 1 day after inoculation; washed immediately.	6	46
B	Check (untreated)	77	99
	5 percent borax 1 day after inoculation; washed after 3 days	23	47
	1 percent phenate 1 day after inoculation; washed immediately.	30	54
	5 percent borax 2 days after inoculation; washed after 3 days	54	83
	1 percent phenate 2 days after inoculation; washed immediately.	19	54
C	Check (untreated)	73	83
	5 percent borax day of inoculation; washed after 1 day	24	39
	1.2 percent phenate day of inoculation; washed immediately.	21	54
	5 percent borax 1 day after inoculation; washed after 1 day	15	34
	1.2 percent phenate 1 day after inoculation; washed immediately.	7	34
	1.2 percent phenate 1 and 3 days after inoculation; washed immediately.	0	0
D	Check (untreated)	49	79
	5 percent borax 1 day after inoculation; washed after 1 day	19	53
	1.2 percent phenate 1 day after inoculation; washed immediately.	3	27

At the end of 1 week there was decidedly more decay of the borax-treated fruit than of the phenate-treated fruit in four out of six possible comparisons, but at the end of 2 weeks this difference had largely disappeared. The longer period of exposure in the case of borax may have influenced the results. It seems probable that the borax may not have been completely washed off after once becoming thoroughly dry and was therefore present to cause inhibition or killing at a later date. It should be noted that particularly good results were obtained in the one instance (table 5, lot C) in which the

phenate treatment was given at the end of 1 day and again at the end of 3 days. There was some indication that in advanced stages of infection phenate treatments were more effective than borax treatments (table 5, lots B and C).

EXPERIMENTS WITH NATURALLY INFECTED FRUIT

In April and May 1939, 1940, and 1941, and in the fall, winter, and spring of the 1941–42 season experiments were carried out at Orlando on naturally infected fruit. It was not possible to separate the decay caused by *Diplodia* from that caused by *Phomopsis* on the basis of general appearance, and, in order to be certain as to the prevailing organism and the relative resistance to control, large numbers of cultures were made in 1940, 1941, and 1942.

Both *Diplodia* and *Phomopsis* were found to be present in abundance in the spring of 1940 and in the fall and winter of 1941–42, but in the spring of 1941 all the decay was due to *Phomopsis*. The spring of 1941 was unusually cool, and this may account for the entire absence of *Diplodia* in the experimental fruit at that time.

ETHYLENE TREATMENTS

Ethylene treatments are considered an almost indispensable seasonal procedure in the preparation of citrus fruit for market, because of the unfavorable impression made by fruit that contains any green color in the rind. Early and midseason oranges do not lose all their green color by the time the fruit is ready for market. To some extent this is also true of the late-ripening varieties and in addition these often regreen before the end of the shipping season. In order to remove the green from the rind, ethylene treatments are given for 1, 2, and sometimes even for 3 and 4 days in rooms in which the temperature is held at 80° to 85° F. and the relative humidity at 87 to 92 percent.

It is well known that these ethylene treatments weaken the fruit and very greatly increase the later development of stem-end rot. When both organisms are present the decay that develops on ethylene-treated fruit is likely to be due almost entirely to *Diplodia*, whereas that which develops on untreated fruit is likely to be due to *Phomopsis (3, 8, 13, 14, 16)*. The effect of ethylene treatment on decay is shown in figure 3, *A*, which gives the average of the results in eight separate experiments in the 1941–42 season. It will be noted that nearly all the increase from ethylene treatment was due to *Diplodia*.

The results naturally raise two questions: (1) What is there in the ethylene treatments that is so much more favorable to *Diplodia* than to *Phomopsis?* (2) Since *Diplodia* can produce decay in half the time required by *Phomopsis*, why does not *Diplodia* cause more decay than *Phomopsis* when the fruit is not treated with ethylene? There are at least three factors that may have a bearing on these questions. The first, naturally, is temperature. The temperature of the ethylene rooms is near the optimum for *Diplodia* and above the optimum for *Phomopsis* (p. 369). This would undoubtedly give *Diplodia* a decided advantage during the period of treatment and for a considerable time thereafter, but it can hardly account for the large increase in *Diplodia* rot even into the third and fourth week of holding.

Another factor to be considered is the aging and weakening effect of ethylene treatments on the buttons. This is undoubtedly favor-
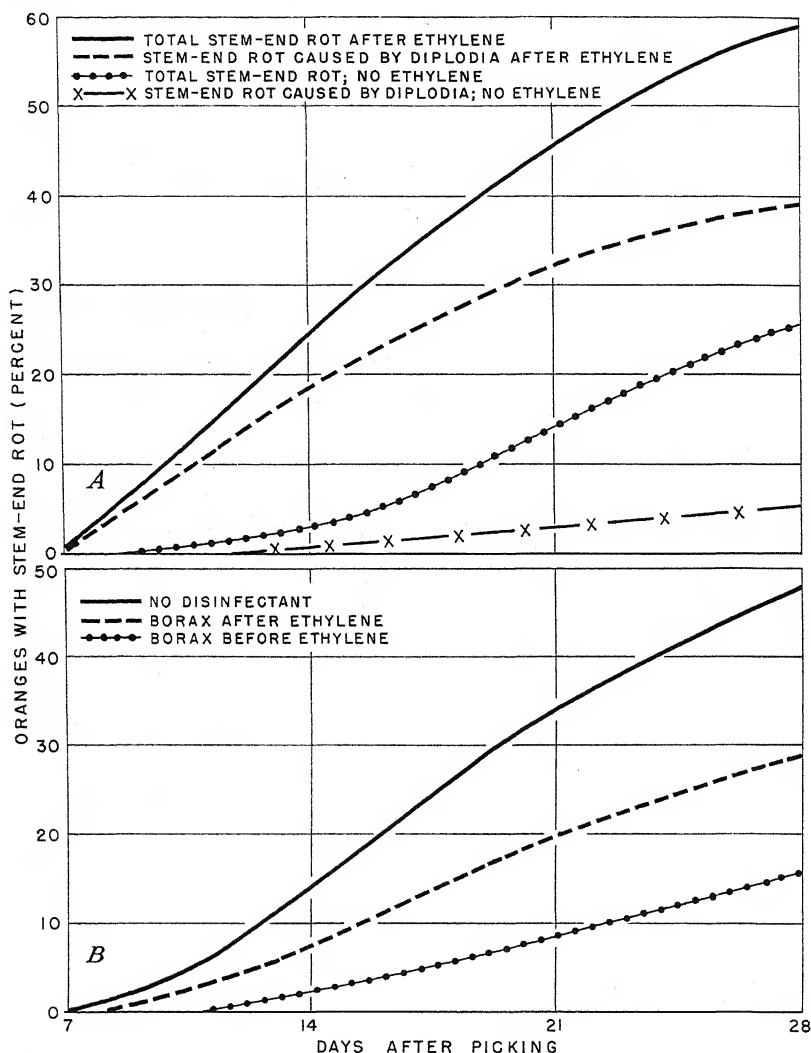
FIGURE 3.—*A*, The effect of 42- to 45-hour ethylene treatments upon stem-end rot and upon the organisms causing it. The curves give the averages for 8 separate experiments carried out in the 1941–42 season—5 with Parson Brown, 2 with Valencia, and 1 with seedling oranges. There was a total of more than 600 oranges held at 70° F. after each treatment. *B*, Stem-end rot as affected by borax treatments before and after 42- to 45-hour ethylene treatments. Average of 2 tests with Valencias in the spring of 1941 and 3 with Parson Browns in the fall of 1941. The borax was applied as an 8-percent solution at 100° F. for 2 minutes; the fruit, which was held at approximately 70° F., was washed 2 days later.

able to a more rapid progress of both organisms, but *Diplodia* is known to be less actively parasitic than *Phomopsis* and is probably more

favored by the aging and weakening of the buttons. It is believed that the effect of ethylene on the buttons is probably the most important factor in the increase in *Diplodia* decay.

A third factor is the effect of ethylene upon spore germination. As stated earlier, the fully mature two-celled spores of *Diplodia* do not germinate freely in water but germination may be stimulated by ethylene. Upon free exposure to the air the one-celled spores soon die or pass over into the two-celled form unless conditions are favorable for immediate germination. In showery weather the fruit might be expected to carry a liberal supply of freshly exuded one-celled *Diplodia* spores, but under average weather conditions it is likely that only the two-celled form would be present and that ethylene would help to start them into activity.

The results shown in figure 3, *A*, however, should not be taken as proof that the ethylene treatment has no effect upon *Phomopsis* decay. In experiments made in the spring of 1941 with fruit that carried no *Diplodia*, it was found that at the end of 2 weeks at 70° F. 21 percent of the ethylene-treated fruit on an average had decayed as compared with 14 percent of the untreated fruit. Apparently the usual dominance of *Diplodia* on ethylene-treated fruit is due not to any inhibition of *Phomopsis* but rather to the naturally more rapid growth of *Diplodia* and the creation of conditions favorable to its development.

FORMALDEHYDE, CHLORINE, AND SODIUM BISULFITE TREATMENTS

Various disinfectants have been tested on naturally infected fruit, but at strengths which caused no injury most of them gave little control of stem-end rot; in fact, stem-end rot was sometimes increased.

Formaldehyde was tested both as a gas and as a liquid. It usually gave reduction in stem-end rot, but never a satisfactory control even when used at strengths that caused injury to the rind.

Chlorine was used as a gas and in the form of sodium hypochlorite solutions. Oranges showed little injury even with extreme treatments, but stem-end rot was increased almost as often as it was decreased. Sodium bisulfite solutions were tested at various strengths but the result was usually an increase in stem-end rot. Increase in stem-end rot resulting from chlorine and sodium bisulfite treatments was apparently due to the chemicals causing greater injury to the buttons than to the fungi, the dead or weakened buttons thus furnishing favorable conditions for the rapid advance of the decay organisms.

BORAX AND SODIUM ORTHO-PHENYLPHENATE TREATMENTS

In the experiments with hand-inoculated fruit borax and sodium ortho-phenylphenate were the only materials tested that gave promise of having commercial value, and they were given many comparative tests on naturally infected oranges.

Effect of Time of Application

As pointed out previously (p. 372), with hand-inoculated oranges there was some indication that phenates were effective at a more advanced stage of infection than borax. The same was true with naturally infected fruit and was especially evident in treatments given before and after ethylene. Figure 3, *B*, presents a comparison

made between borax treatments before and after the fruit had passed through the coloring room. During the first 3 weeks of holding there was more than twice as much stem-end rot on the oranges treated after ethylene as on those treated before ethylene.

The importance of applying borax as soon after picking as possible and the benefits of applying it before ethylene treatment have been repeatedly emphasized by Winston (*13, 15, 16*) and others.

A similar series of experiments was made in which phenate was applied as a 1.2-percent solution at 100° F. for 2 minutes and the fruit was washed immediately. At the end of 2 weeks in three tests with Parson Browns, four with Valencias, and two with Jaffas an average of 2.1 percent of the oranges treated after ethylene had stem-end rot as compared with 3.3 percent of those treated before ethylene and 11.8 percent of the untreated ones. In contrast with the results with borax, the phenate treatments after ethylene made a somewhat better showing in decay control than the treatments before ethylene.

The contrast in the results with the two fungicides is probably due partly to the difference in methods of application. The borax was left on the fruit for 2 days and the phenate for only 2 minutes. It has been suggested that the fungi had penetrated too deep into the button tissue during the ethylene treatment to be reached by borax, and the curves of figure 3, *B*, seem to give some support to this idea; yet the failure to control with the after-ethylene treatment was nearly as great with the rots appearing at the end of 3 and 4 weeks as with those appearing at the end of 9 to 14 days. It seems probable that much of the advantage of applying borax before ethylene is due to the greater penetration of the chemical into both fungus and button tissue under the high temperature and high humidity of the ethylene room and the less complete removal in the later washing.

The results with phenate indicate that the decay organisms had not penetrated too deep to be reached by a disinfectant at least after 42 to 45 hours in the ethylene room. In fact, fruit on which the phenate treatment was delayed after removal from the ethylene room often showed less decay than fruit treated immediately after removal. An example of this is shown in table 6. In this case the results from disinfecting 1, 2, or 3 days after removal from the ethylene room were better than those from disinfecting immediately after removal.

TABLE 6.—*Stem-end rot of Parson Brown oranges held at 70° F. as affected by delays in and repetitions of phenate treatment after a 42-hour period in the ethylene room*

[Phenate applied as a 1.2-percent solution at 100° F. for 2 minutes; fruit washed immediately]

Lot	Treatment	Fruit with stem-end rot—		
		10 days after harvest	12 days after harvest	14 days after harvest
		Percent	*Percent*	*Percent*
A	Check (untreated)	1	8	20
B	Phenate immediately after removal from ethylene room	1	2	11
C	Phenate 1 day after removal from ethylene room	0	0	4
D	Phenate 2 days after removal from ethylene room	0	0	2
E	Phenate 3 days after removal from ethylene room	1	1	2
F	Phenate immediately and 1, 2, and 3 days after removal from ethylene room	0	0	0

This apparent advantage from delaying the phenate treatments, however, is based on records of decay at a given number of days after harvest. If the table were changed to show the percentage of stem-end rot a given number of days after the disinfecting treatment the advantage of the delayed treatments would largely disappear. From the practical point of view, the important fact is that phenate treatments were fairly effective even when there was considerable delay in applying them. The phenate as applied apparently had an inhibiting rather than a killing effect. After 2 or more weeks at 70° F. the organisms on the treated fruit again became active. This was avoided by a daily repetition of the disinfecting treatment as shown in lot F (table 6), a method that of course would be impracticable under most commercial conditions.

EFFECTIVENSS OF VARIOUS TREATMENTS

The foregoing results make it evident that the data from ethylene-treated fruit and untreated fruit should be considered separately and also that phenate treatments after ethylene are likely to be of more practical value than phenate treatments before ethylene.

Table 7 gives further data on the relation of ethylene and phenate treatments to the development of stem-end rot. Phenate-treated oranges that had received ethylene had approximately the same amount of stem-end rot as the untreated fruit that had not received ethylene.

TABLE 7.—*Stem-end rot of oranges held at 70° F. as affected by treatment with phenate*

[Phenate applied as a 1.2-percent solution at 100° F. for 2 minutes 2 days after harvest; fruit washed immediately. Average of 5 separate experiments (2 with Valencia and 1 each with Parson Browns, seedlings, and Jaffas). Ethylene treatment for 42 to 45 hours]

Lot	Treatment	Fruit with stem-end rot—			
		9 days after harvest	14 days after harvest	17 days after harvest	21 days after harvest
		Percent	*Percent*	*Percent*	*Percent*
A	Ethylene treated, no disinfectant	3	12	29	45
B	Phenate after ethylene	1	3	8	15
C	No ethylene, no disinfectant	0	3	9	20
D	Phenate, no ethylene	0	0	0	4

With fruit that had not been in the coloring room the disinfecting treatment delayed the development of stem-end rot approximately 1 week and with fruit that had received ethylene there was a corresponding delay of approximately half a week. Any lowering of the temperature below 70° F. would increase the spread in time. Under the usual shipping conditions the lower temperatures probably would double these time periods.

The control obtained with phenate after ethylene was not as complete as that shown in figure 3, *B*, for borax before ethylene, but it was better than that obtained with borax after ethylene. The treatments reported in table 7 did not cause injury; yet it was found that a 1.2-percent solution would sometimes cause rind injury if the temperature rose much above 100° F. or if the treatment was not followed by thorough washing. It seemed evident that 100° should

be regarded as a maximum temperature for safe operation with this strength of solution.

Various modifications in the phenate applications were tested. At 120° F. injury was obtained with 0.8- and 0.5-percent solutions and the decay control was poorer than with a 1.2-percent solution at 100°. Increasing the temperature with a particular strength of solution increased injury more rapidly than decay control. The most satisfactory results were obtained at 100° or lower. Increasing the period of treatment seemed to be favorable to decay control without greatly increasing injury.

In view of these observations tests were made at lower temperatures and under conditions that seemed likely to extend the period that the disinfecting agent would be active. In some cases the phenate treatments were followed immediately by dipping in a water-wax emulsion and in others the phenate was added to the water phase of the wax emulsion. The results of these experiments are shown in table 8.

TABLE 8.—*Stem-end rot of oranges as affected by various 2-minute treatments*

All lots except B4 run in duplicate. A and B, Seedling oranges stored at 65° F.: A, 42-hour ethylene treatment after borax and before phenate; B, no ethylene. C, Valencia oranges stored at 80° F.: 1 and 2, no ethylene; 3 to 10, inclusive, ethylene; 4 and 5, treatments before ethylene; 6 to 10, treatments after ethylene]

Lot		Treatment	Fruit with stem-end rot—				
			11 days after harvest	14 days after harvest	17 days after harvest	21 days after harvest	28 days after harvest
			Percent	*Percent*	*Percent*	*Percent*	*Percent*
A	1	Check (no disinfectant)	5	24	44		
	2	8 percent borax at 100° F.; washed after 2 days	2	4	10		
	3	1.2 percent phenate at 100° F.; washed immediately	2	7	17		
	4	1.2 percent phenate at 100° F.; then wax emulsion	0	3	18		
	5	2.0 percent phenate at 80° F.; washed immediately	0	3	18		
	6	2.0 percent phenate at 80° F.; then wax emulsion	0	6	16		
B	1	Check (no disinfectant)	0	1	8	14	28
	2	1.2 percent phenate at 100° F.; washed immediately	0	0	1	1	6
	3	1.2 percent phenate in wax emulsion at 100° F	0	0	0	0	5
	4	1.2 percent phenate in wax emulsion at 100° F. (dipped only)	0	0	0	0	5
	1	Check (no disinfectant)	1	4	9	16	
	2	1.2 percent phenate at 100° F.; washed immediately	1	1	1	4	
C	3	Check (no disinfectant)	4	11	22	40	
	4	8 percent borax at 100° F.; washed after 2 days	1	2	4	7	
	5	1.2 percent phenate at 100° F.; washed immediately	1	4	12	29	
	6	1.2 percent phenate at 100° F.; washed immediately	0	0	3	18	
	7	1.2 percent phenate at 90° F.; washed immediately	0	0	3	18	
	8	1.2 percent phenate at 100° F.; then wax emulsion	0	1	4	17	
	9	1.2 percent phenate at 90° F.; then wax emulsion	1	2	5	20	
	10	1.2 percent phenate in wax emulsion at 100° F	1	1	2	13	

A 2.0-percent solution at 80° F. gave slightly better results than a 1.2-percent solution at 100°. A 1.2-percent solution gave practically

as good control at 90° as at 100°. Following the phenate treatments with immersion in a water-wax emulsion caused no injury and gave as good control as following them with washing. Including the phenate in the water-wax emulsion caused no injury and in the few times tested it gave slight y better control than any other method.

The phenate treatments after ethylene were as satisfactory as borax before ethylene during the first 2 weeks at 70° F., but they failed to give as good control in long holding.

Three different water-wax emulsions were used in the experiments; two of them had a relatively high percentage of carnauba wax and in one the wax was largely paraffin. As there was little difference in disease control, the results with the different waxes have been combined to give the data of table 8.

Cultures were made throughout these and other disinfection experiments to determine whether *Diplodia* and *Phomopsis* were being equally controlled. Treatment with either borax or phenate before ethylene gave far better control of *Diplodia* than of *Phomopsis*. Water applications of phenate after ethylene usually gave somewhat better control of *Diplodia* than of *Phomopsis*, but when the phenate was added to a wax emulsion there seemed to be no difference in the control of the two organisms.

PRACTICAL CONSIDERATIONS

Stem-end rot is not ordinarily the cause of heavy losses during the first week after picking, a period covering the usual shipping and wholesale market operations, but it often causes heavy losses during the second week after picking when the fruit is in the hands of the retailer or consumer. These losses are borne largely by the consumer either directly or indirectly, but shippers and growers also lose because of sales resistance to their product.

The extent of this loss from decay is modified greatly by packing-house treatment. Ethylene-treated fruit is much more likely to decay than nontreated fruit, and much can be accomplished in the control of decay by not using the treatment on fruit that does not have enough green in the rind to be definitely objectionable. For further control at least two disinfectants, borax and sodium ortho-phenylphenate, are available.

For ethylene-treated fruit borax is the most effective disinfectant provided the application is made before the ethylene treatment and the borax is left on the fruit during the treatment. Pre-ethylene treatment has the disadvantage of requiring an extra operation.

As a treatment after ethylene or on fruit that has not received ethylene sodium ortho-phenylphenate has been found effective, at least up to the fourteenth day after picking, with fruit held at 70° F. With the usual temperatures in transit and on the market, this period of protection would be considerably extended. Careful control of temperature and of concentration is required to prevent red specking of the rind.

SUMMARY

Methods are reported for obtaining a continuous supply of *Phomopsis* spores and of one- and two-celled *Diplodia* spores in culture.

Evidence is presented showing that the stem-end rot fungi can gain ready entrance to the fruit either through the cut on the stem or through other parts of the button.

Diplodia was found to produce decay in less than half the time required by *Phomopsis*, even at temperatures somewhat more favorable to *Phomopsis*.

Oranges that were held in an ethylene room for 42 to 45 hours at a temperature of 80° to 85° F. and a relative humidity of 87 to 92 percent were compared with similar oranges that were held for the same period in a basement at a temperature of approximately 70° and a relative humidity of 80 to 90 percent. At the end of 2 weeks after harvest the ethylene-treated fruit had about 9 times as much stem-end rot as the untreated fruit and at the end of 3 weeks more than 3 times as much. This increase was due almost entirely to *Diplodia*.

Three possible reasons are suggested for the increased decay after ethylene treatment: The higher temperatures of the ethylene room, the aging and weakening of the buttons, and a possible stimulation of the germination of *Diplodia* spores. Of these the effect upon the buttons is considered the most important.

Formaldehyde, calcium hypochlorite, sodium hypochlorite, and sodium bisulfite failed to show promise as disinfectants for the prevention of stem-end rot. Of various disinfectants tested borax and sodium ortho-phenylphenate gave the best results.

Applications of borax made after a 42- to 45-hour ethylene treatment were less than half as effective as applications made before the ethylene treatment.

Applications of sodium ortho-phenylphenate after ethylene were fully as effective as those made before ethylene.

The phenate treatments after ethylene were as effective as borax treatments before ethylene during the first 2 weeks in 70° F. storage but failed to give as good control of stem-end rot during long holding.

The phenate was used as a 1.2-percent solution at 100° F. and caused no injury when followed by thorough washing, but increases in temperature resulted in injury with this strength of solution. Nearly as good decay control was obtained at 90° as at 100° with a 1.2-percent solution, indicating that in practical operations satisfactory results should be obtained by holding the temperature in the nineties. Increasing the temperature with a particular strength of phenate solution resulted in a greater increase in injury than in decay control.

Including 1.2-percent of phenate in the water phase of a water-wax emulsion at 100° F. caused no injury, and in the few times tested gave better control of stem-end rot than any other method in which this material was used.

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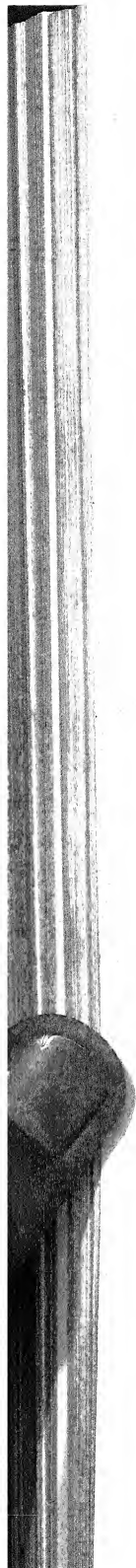
A STUDY OF THE SWEET-AND-SOUR APPLE CHIMERA AND ITS CLONAL SIGNIFICANCE [1]

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INTRODUCTION

The Sweet-and-Sour apple, a form characterized by having some sour and some sweet tissue, has been known to pomologists for well over a century and a half. A contributor (*4*)[3] to the Rural New Yorker in 1897 states that 50 years earlier, in 1847, he picked 12 barrels of fruit from such a tree in an orchard near Barton Center, N. Y., where it was known as the Compound apple. E[liott] (*8*) reports the variety as having been cataloged by nurserymen as early as 1794. Numerous references to an apple variety of this description are found in the literature between 1840 and 1900, though there is no indication that it was valued or grown as anything other than a curiosity.

ORIGIN AND HISTORY

Evidently the Sweet-and-Sour apple is a variety, or type, of multiple origin, for, though cataloged at different times by a number of nurseries, it is not at all clear that they referred to the same variety. Many, if not most, of the brief descriptions imply that the single tree to which reference was being made had just appeared spontaneously, or traced back to such a single tree in some older orchard. Thus there is the record of a single tree in the orchard of Wm. T. Hamilton, Clifton Park, N. Y., though this tree was grown from scions set about 1805 (*20*). A. S. Moss (*17*), of Fredonia, N. Y., in 1855 reported having a tree bearing sweet-and-sour fruits; it is described as a seedling on which "Greening" scions had been set. Barry (*3*) reports growing such a variety for a number of years, propagated from a limb sport on a Greening tree which had been under his observation for 30 years. Besides this particular sweet-and-sour limb sport which he propagated, he reported knowing of several others.

A limb sport of this same type on an old Rhode Island Greening tree in an orchard near Breedsville, Mich., was brought to the writer's attention in 1927 and propagated by him. In 1932 scions were obtained from a similar limb sport in the orchard of D. D. Gordon, of Rushford, N. Y. This Rushford, N. Y., tree was propagated as Rhode Island Greening Selection No. 573, and its progeny furnished most of the material on which this report is based.

However, sweet-and-sour apples have not all been sports from Rhode Island Greening or from other varieties of the Greening type.

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L'Hermès (*14*) has reported such a combination of red acid and yellow sweet types that was known to have originated as a seedling. Gaudichaud (*10*) has reported a similar combination of a reinette rousse and a reinette canada jaunâtre and Castle (*6*) one showing the characteristics of Boston Stripe and Golden Russet.

That many of these sweet-and-sour apples originated as limb sports there can be no doubt. Presumably most, if not all, originated either as limb or as seedling sports, though many of the early accounts describing them or telling about their occurrence suggest that some may have originated as graft hybrids developing at the point of union between stock and scion where a Greening scion was set on a sweet apple stock, or vice versa. It is significant, however, that in no instance is the evidence clear enough definitely to assign one of these sweet-and-sour forms to graft hybrid origin.

SWEET-AND-SOUR TREE AND FRUIT CHARACTERISTICS

The one distinctive characteristic of the Sweet-and-Sour variety, or type, is that the individual fruits are partly sweet and partly sour. Usually the sweet and sour portions have been described as consisting of alternating segments, of varying width, extending from base to apex, the sour sections being green in color and the sweet sections yellow (*4, 18, 1*). Some of the descriptions emphasize the fact that characteristically there is a clear line of demarcation between the two kinds of tissue (*16*) and that often the green, sour segments are raised above the yellow, sweet sections, giving rise to alternating ridges and hollows on the surface of the fruit. Thus the general concept of the Sweet-and-Sour apple has been that of a sectorial chimera. Other tissue patterns, however, have been observed. Avery (*2, p. 88*) records such a variation in pattern in the following words: * * * "upon the same apple there would be little spots about the size of the end of my finger that would be very sour, indeed, and usually these sour parts are green." In the sweet-and-sour combination described by Castle (*6*), and likewise in one cited by Darwin (*7, v. 1, p. 425–426*), the division lines between the sweet-and-sour tissues were at right angles with, rather than parallel to, the axis of the fruits.

Reversion to the all-sweet or all-sour condition has been frequently reported. Thus Avery (*2, p. 88*) reports: * * * "I have seen apples growing on the same limb, one would be sour and the other entirely sweet." Moss (*17*) states: "From half a bushel to one bushel [out of 10 to 12 bushels borne by the tree] will be sweet, not as large but nearly the color of Greenings, but more golden when ripe, which is earlier than the Greenings. These sweet apples * * * grow interspersed on different spurs on the same limbs with the Greenings." When such complete reversion takes place, it would be expected that scions taken for propagation purposes might produce some trees that would show complete reversion to the sour or the sweet type. Evidently this has occurred more or less frequently. One contributor (*11*) to the Rural New Yorker writes: "Several times I have had grafts sent me of the so-called sweet-and-sour apple, but on my trees, they all reverted to sour, ever anything else." Such was the experience of the author when he propagated the sweet-and-sour limb sport found near Breedsville, Mich., in 1927. Four trees of that strain have borne heavy crops for several years, all of the Rhode Island Greening type. A

somewhat more unusual type or pattern of reversion is described by Morse (*16*) as follows: "One year, the tree bore, to all appearances, only Rhode Island Greenings; the next year, some were part sweet and part sour, some all sweet and some all sour, and all these different kinds might be growing on the same branch * * * A year or two after, the tree bore a heavy crop of fine sweet apples; not one sour or mixed apple could be found. I never knew what the tree would produce until the crop presented itself." This rather strange performance is similar to one recorded for a russet sport on the Stark variety of apple, described by Swanson and Gardner (*19*).

MATERIALS USED IN THIS STUDY

Scions from the limb sport found near Breedsville, Mich., produced trees that yielded fruits exclusively of the green-skinned, sour-fleshed Rhode Island Greening type, thus showing complete reversion. Scions from the Rushford, N. Y., tree, on the other hand, produced trees that yielded sweet-and-sour fruits that, at least superficially, were like those described in the literature of two, three, and four generations ago. A number of nursery trees of this selection (No. 573) were grown; six were later set in the orchard. Their first fruits were borne in 1939. The crops were light and did not furnish enough material for a thorough study of the segregation, reversion, and peculiarities of distribution of sweet-and-sour tissue that were more or less evident. Rather careful examination, however, suggested that here is a chimera seemingly unique, a study of which might throw some light on the fundamental nature of the many horticultural varieties that belong in this general class or group.

OBJECTIVES

The specific objectives in undertaking the studies made in 1940–42 were: (1) To determine the pattern or patterns of distribution of tissues of different kinds—in this instance sweet and sour—in a supposedly sectorial chimera, (2) to determine the nature and range of distribution in sweetness or sourness of fruit borne by a single tree, (3) to determine the constancy of this range from year to year, and (4) to measure individual tree differences in respect to amount or degree of reversion and segregation.

In view of the fact that the apple is a pome fruit that includes a considerable variety of tissues—from pith to epidermis according to some students of morphology, only ovarian and floral envelope tissues according to others—it was thought that the study might throw new light on the depth of penetration of chimeral tissue mosaics.

METHODS OF PROCEDURE

Studies in 1940 were limited to field notes and observations on each of the apples borne by each of the six trees. Records were made of the amounts and distribution of sweet and sour tissue, as indicated by surface coloring of the fruit and by the width and size of the ridges, hollows, knobs, and depressions that showed differences in color. These observations were checked by organoleptic tests.

Similar records were made on the 1941 crops. It became evident, however, that more exact methods should be employed to determine the

distribution of the tissues of various degrees of sourness and sweetness within the apple. As a result of a number of preliminary tests it was found that a 0.4-percent aqueous solution of the sodium salt of bromcresol green gave a sharp color reaction to the various amounts of acidity present in the sweet-and-sour apples of this strain. By painting this solution on a fresh slice of fruit with the aid of a camel's-hair brush the acid portions of the tissue changed to a light yellow or orange yellow and the sweet tissues to a deep blue, within a few seconds. These color changes remained constant for a period of several hours so that they could be photographed. By promptly pressing the colored surfaces of the slices against a soft-textured sheet of paper its color record was readily transferred. The areas of sweet and sour tissue could then be measured with a planimeter and accurate quantitative records obtained. A number of fruits of the 1941 crop and all of the fruits of the 1942 crop were studied in this way.

PRESENTATION OF DATA

OBSERVATIONS ON 1940 AND 1941 CROPS

Observations were made in 1940 and 1941 on the distribution of fruits of various degrees of sweetness or sourness, on each of the several trees; the results are summarized in table 1. Though only four trees bore fruit in 1940 and five in 1941, certain individual tree differences were noted. Trees D and E showed a marked tendency to produce either all-sweet or predominantly sweet fruits; on the other hand, trees F and G showed an equally marked tendency to produce either all-sour or predominantly sour fruits

TABLE 1.—*Notes on tree differences in distribution of fruits possessing various proportions of sweet and sour tissue in 1940 and 1941*

Tree	Tree differences in fruit distribution for year—	
	1940	1941
D	18 fruits. 16 were all-yellow early ripening, *apparently* all-sweet. 2 were ribbed, with alternating green and yellow ribs and hollows, the green sections sour; later ripening by a week than the sweet fruits.	Yield ½ bushel. Nearly all fruits were early maturing, about 2 weeks ahead of Rhode Island Greening; small in size, comparable to Tolman Sweet. Nearly all appeared to be uniformly sweet, yellow, and regular in outline. A few had narrow raised ridges of greener, semi-acid tissue; however, in these the all-sweet tissue predominated.
E	9 fruits. 7 were all-yellow, early ripening, apparently all-sweet. 1 was early ripening and all-yellow and all-sweet, except for several green spots or knobs at the calyx end, these knobs being green and sour. 1 was late maturing (with Rhode Island Greening) and *apparently* all-green and all-sour.	Yield ¼ bushel. Fruits variable; those on certain branches *apparently* were small, all-yellow, and all-sweet; those on other branches were later maturing, ribbed, the ribs being green and sour and the hollows yellow and sweet, the sweet sectors generally being wider than the sour sectors.
F	34 fruits. 33 were all-yellow, early ripening, *apparently* all-sweet. 1 was later maturing and was all-yellow and all-sweet, except for a single green, sour sector extending from stem to calyx.	Yield ½ bushel. Fruits variable; on the average somewhat larger in size than the fruits of tree E and later in maturing, with wider green sour segments.
G	12 fruits. All were late maturing (with Rhode Island Greening). Most *appeared* to be symmetrical, all-green and all-sour. Several had narrow yellow, sweet sectors, but the green sour tissue in them predominated.	Yield ⅛ bushel. Fruits variable; those on certain branches *appeared* to be all-green, all-sour; those on other branches, the majority, were ribbed, with the ribs green and sour, the hollows yellow and sweet, and the ribs predominating over the hollows.
H	No fruits	Yield ¼ bushel. Fruits variable; many all-green; in the others green predominated.
I	do	No fruits.

DISTRIBUTION OF SWEET AND SOUR TISSUE

Cross, longitudinal, and tangential sections were made through a number of representative fruits from each of the trees of the Sweet-and-Sour -variety. Sections were also made of Rhode Island Greening fruits to serve for comparison. The sections were stained as described under Methods of Procedure, and then photographed. A representative series of these photographs is presented in figures 1–6.

It will be noted that the stained cross sections of the acid to subacid Rhode Island Greening fruits (fig. 1) show some variation in degree of acidity, a variation that is associated with degree of maturity. For instance, of the two fruits on the right, the upper one was somewhat less mature than the lower one, but both show a comparatively uniform coloration from surface to center and from side to side. Slight differences in the intensity of the staining, associated with corresponding differences in amount of bromcresol green, account for part of the difference in the density of shading seen in the illustration.

Figures 2 and 3 show the distribution of sweet and sour tissue in median transverse sections of each of four representative fruits from two trees of the Sweet-and-Sour apple. They serve to emphasize the point brought out in table 1 that no two trees of this variety are alike in respect to amounts or distribution of sweet and sour tissues, except those that have more or less completely reverted to an all-sweet or an all-sour type. Indeed, they show that no two fruits on the same tree are exactly alike. When taken along with figures 4, 5, and 6, showing cross sections at different levels from the same fruit, and also radial and tangential sections, they indicate an extreme irregularity in the distribution of the two kinds of tissue within the fruit—a distribution that is more properly described as being without pattern than as having pattern. There are segments or radial sectors of sweet tissue alternating with similar segments or radial sectors of sour tissue; there are also surface patches or masses of the one subtended by larger masses of the other; there are similar islands of the one type embedded in larger masses of the other; the calyx end may be like or unlike the stem end of the same fruit; there is almost as much lack of radial as of bilateral symmetry. Certainly the distribution is not such as corresponds to current concepts of sectorial, periclinal, or even mericlinal chimeras.

Another characteristic, perhaps suggested but not definitely established by surface color patterns and organoleptic tests, clearly brought out by these illustrations, is that for the most part the boundary lines between areas or masses of sweet and sour tissue are not sharp. Seldom can an exact line be drawn between them. Instead there is a zone, sometimes narrow, sometimes wide, where there is a mixture of the two kinds of tissue. Sometimes these areas of mixed tissue are principally of the one kind or of the other; sometimes the mixture consists of about equal parts of the two components. In some instances the mixture constitutes a relatively large part, in others a small part, of the volume or mass of the fruit.
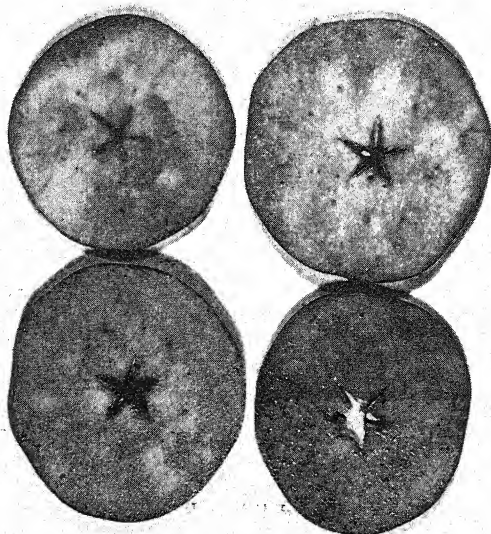
FIGURE 1.—Cross sections through the centers of four average Rhode Island Greening fruits, characterized by sour, or at least subacid, tissue, treated with a bromcresol-green solution, which stains acid tissue yellow and sweet tissue blue. Note the uniform acid tissue, indicated by the light color.
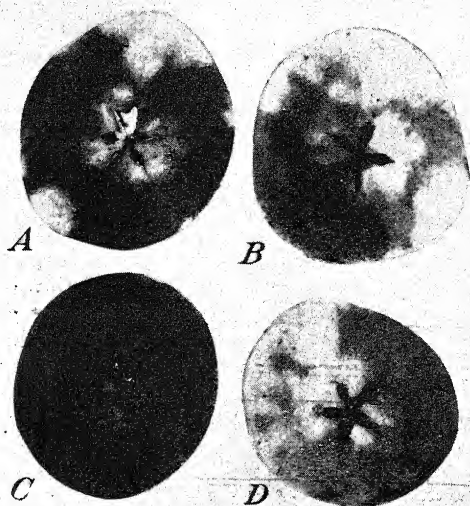
FIGURE 2.—Cross sections through the centers of four representative Sweet-and-Sour fruits from tree F, treated with a bromcresol-green solution. Note that fruit *C* is all sweet, fruit *A* more sweet than sour, and fruits *B* and *D* more sour than sweet. Also note the very irregular distribution of the two kinds of tissue.
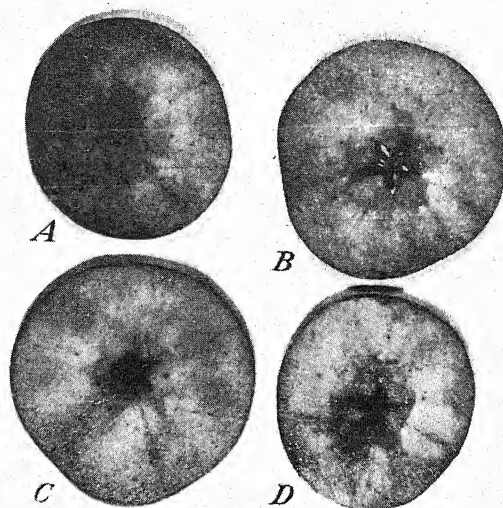
FIGURE 3.—Cross sections through the centers of four representative Sweet-and-Sour fruits from tree G, treated with a bromcresol-green solution. Note that the fruit *A* is a third to a half sweet, while *B*, *C*, and *D* are almost all-sour, though they show small radial segments or centrally located patches of subacid tissue.
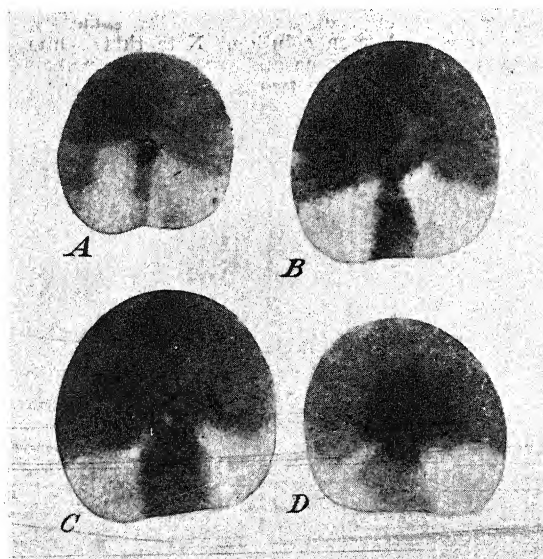
FIGURE 4.—Four cross sections, at different levels, through the same Sweet-and-Sour apple from tree D. Section *A* is from near the calyx end, *B* from just a little above its center, *C* from a little below its center, and *D* from near the stem end. Note that a larger part of the basal end of this fruit is acid than of the apical end.
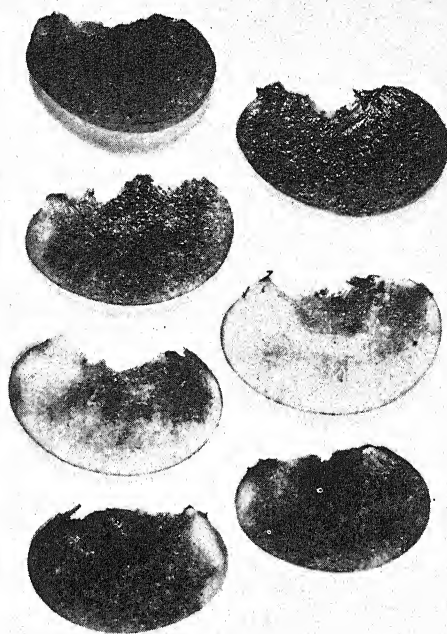
FIGURE 5.—A series of radial sections through the same apple from tree F, treated with a bromcresol-green solution. Note that, while five of the sections show principally sweet tissue and two of them principally sour tissue, the pattern of distribution of the two kinds of tissue is very irregular.
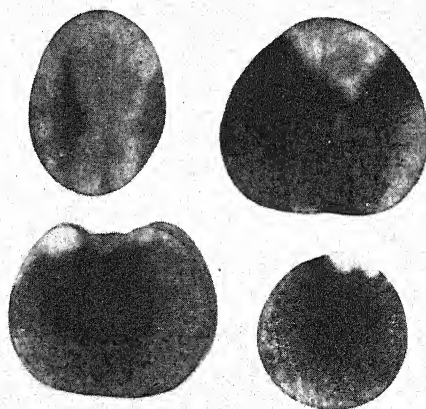
FIGURE 6.—Four vertical tangential sections through a single Sweet-and-Sour apple treated with a bromcresol-green solution. Note the very marked differences between the two sides of the apple.

QUANTITATIVE STUDIES OF THE SWEET AND SOUR TISSUES

The 1942 season crops borne by the six trees of Sweet-and-Sour Selection No. 573 were large enough so that quantitative studies could be made of the amounts of sweet and sour tissue in the fruits from different trees and statistical tests for significance applied to the data that were obtained. Every fruit of the 1942 crop was sectioned transversely through the center, the exposed surface was promptly treated with the bromcresol-green solution, and a few seconds later a print was made showing color, and therefore sweet and sour, distribution over its entire surface. Similar sections and prints were made of the apical and basal regions of each apple. Lines were then drawn on the prints that served as division lines between areas of sour and sweet tissue. These were necessarily arbitrary. The arbitrary lines that were drawn, however, represent very closely where the one or the other kind of tissue dominated over the other. Sweet and sour areas of these cross sections were then measured with a planimeter. Representative data obtained from these planimeter measurements are presented in table 2 and a summary of all the data obtained in table 3.

TABLE 2.—*Percentages of sour tissue in a representative sample of individual fruits from tree D, as indicated by planimeter measurements of sour areas in basal, median, and apical cross sections; 1942 season*

Basal section			Median section			Apical section			Average sour area
Total area	Sour area		Total area	Sour area		Total area	Sour area		
Sq. in.	*Sq. in.*	*Pct.*	*Sq. in.*	*Sq. in.*	*Pct.*	*Sq. in.*	*Sq. in.*	*Pct.*	*Pct.*
4.60	0	0	5.70	0	0	4.50	0.15	0.3	0.1
5.40	0	0	6.20	0.65	10	5.30	.45	8.5	6.2
4.60	0.50	11	5.10	.85	17	4.05	1.80	44	24
4.90	.30	6	6.10	.15	2	4.75	0	0	2.7
4.50	.10	2	5.40	.70	13	4.10	.45	11	8.7
5.20	1.30	25	5.75	2.45	43	4.60	2.50	54	40.7
5.70	0	0	6.35	.30	4.8	5.30	.40	7.6	4.1
5.30	1.70	32	6.25	2.40	38	5.10	2.85	56	42
3.50	.15	4	3.90	.55	14	3.40	.25	7	8.4
4.40	1.30	29	5.30	1.20	23	3.70	.95	26	26
5.50	.40	7	6.35	.15	2.4	5.35	0	0	3.1
Average		10.5			15.2			19.4	15.0

TABLE 3.—*Percentages of sour tissue in the fruits from the six different trees, as indicated by planimeter measurements of sour areas in basal, median, and apical cross sections; 1942 season*

Tree	Fruits	Sour tissue in—						Average sour area
		Basal section		Median section		Apical section		
		Range	Average	Range	Average	Range	Average	
	No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
D	73	0–97	21	0–95	22	0–79	23	22
E	26	0–47	22	0–100	19	0–100	22	21
F	144	0–100	20	0–100	16	0–100	22	19
G	29	0–100	98	7–100	97	59–100	98	98
H	99	0–100	30	0–100	35	0–100	34	33
I	4	4–100	70	14–100	52	6–100	58	60

In general, the records substantiate the observations that had been made in 1940 and 1941, some of which are summarized in table 1 and others of which are presented graphically in figures 2–6. They clearly establish the marked differences between different trees of this strain in the relative amounts of sweet and sour tissue in their fruits, the extreme variation among different fruits on the same tree, and the great variations in distribution of sweet and sour tissue within the fruit itself. On the other hand, they do not bear out the impression that was gained from a more superficial examination of the fruits and from organoleptic tests that there are many fruits that show complete reversion or segregation to the sweet or to the sour condition. As a matter of fact only 4 fruits, out of the 375 borne in 1942, showed no trace of sour tissue in any of their cross sections; only 34 fruits (approximately 9 percent) showed no trace of sweet tissue in any of their cross sections. Had a larger number of sections been made of these fruits, it is possible that small islands of tissue of the other type would have been discovered, and the number of fruits classified as segregates correspondingly reduced.

DISCUSSION

Though most of the accounts of the Sweet-and-Sour apple appearing in the literature, as well as casual observations and organoleptic tests of the fruits, suggest that the variety or type should be classified as a sectorial chimera, the data obtained from this study indicate clearly that this is not such a type. Extremely few, if any, of the fruits of the 1942 crop, studied by the staining method described, even closely approximate sectorial chimeras in their pattern of arrangement and distribution of the two kinds of tissue that are present. Nor do they correspond to Baur's concept (*5*) of such periclinal chimeras as *Cytisus adami*, obtained as a graft hybrid between *Cytisus purpureus* and *Laburnum vulgare*, or the *Solanum lycopersicum* × *S. nigrum* graft hybrid of Winkler, though in their partial and complete segregation or reversion to the component parts they more or less resemble them. They more closely resemble the concept of incomplete periclinal or mericlinal chimeras of Jørgensen and Crane (*12*) which accounts for more or less superficial patches or islands of tissue of one kind surrounded by or embedded in tissue of another kind, or perhaps the "mixed or mosaic" chimeral condition of the Golden Buckeye orange and certain other citrus fruits described by Frost (*9*). The latter interpretation is more tenable if one accepts the "appendicular" concept of the morphology of the apple fruit set forth in some detail by MacDaniels (*15*) and concurred in by many others—viz, that the fleshy tissues of the pome fruit are carpellary, being foliar in origin. According to the concept interior core tissue can be interpreted as the exterior layers of much of the fleshy part of an "inverted" xylem-phloem arrangement. As a matter of fact the data presented here lend support to this interpretation of pome tissue origin and arrangement. On the other hand, if one accepts the concept of Kraus (*13*) and many others that the core of the fruit is stelar or toral in origin and is in reality pith tissue, our ideas as to the superficial character of chimeras should be modified, for clearly both sweet and sour tissues are found to

weave many diverse patterns in the core portion of the Sweet-and-Sour apple (see especially figs. 2–4). Whichever the interpretation, the Sweet-and-Sour apple illustrates the fact that the intimate association of two kinds of tissue in the same plant organ may give rise to tissue patterns extending radially to the very center of morphologically complex parts that are as varied as the superficial color patterns of any variegated plant.

It has been pointed out that the name Sweet-and-Sour has been applied to a number of different forms of different origin. Consequently, as it stands today, Sweet-and-Sour is a type, rather than a variety, name. However, when any one of these forms is propagated vegetatively, as a number have been, the resulting group of plants is to be classed as a clonal variety. In the case of this particular variety (No. 573) and apparently in a number of the others of the same type and designated by the same name, a greater diversity is encountered than is associated with the general concept of a clonal variety. It is greater than has commonly been associated with variegated or other chimeral forms. Individual tree differences within the variety were found to be much greater than those between many more or less similar varieties—e. g., varieties belonging to the Greening or the Russet groups. Perhaps many of the variegated and chimeral forms that are now being grown would be found to have an all-pervading mixture of the genetically (?) distinct component parts, were a technique available to differentiate between them comparable to the one employed in this study.

How well or faithfully such individual tree differences can be perpetuated by special care in the selection of wood or buds for propagation cannot be predicted from this study. If, as there is accumulating evidence to indicate, a much larger proportion of our clonal varieties than have heretofore been recognized as such, are in reality chimeras, the matter becomes one of practical importance rather than just academic interest.

SUMMARY

The general characteristics of the Sweet-and-Sour apple are described as they are reported in the literature of the past 150 years and as they appeared on trees of this type in an experimental orchard.

Clear differentiation between sweet and sour tissue in the same fruit was made possible by treating an exposed section to a 0.4-percent aqueous bromcresol-green solution.

All of the trees proved to be chimeras, producing partly sweet and partly sour fruits.

Some of the trees produced fruits in which the sweet tissue predominated over the sour; other trees produced fruits in which the sour tissue predominated over the sweet. These tree differences remained constant from year to year.

Cross sections of the same fruits at different levels and also tangential and radial sections of the same fruit show extreme diversity in the sweet and sour tissue pattern or distribution.

If the Sweet-and-Sour apple is classified as a mericlinal or mosaic type of chimera, this concept implies that the pome fruit is essentially "appendicular" in its morphology.

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EFFECTS OF HARVEST DATE AND CURING ON THE COMPOSITION AND PALATABILITY OF PECAN NUTS[1]

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INTRODUCTION

Pecan nuts harvested at relatively early stages of maturity, it has been noticed, are nearly identical in flavor after curing with riper nuts harvested and cured at later dates. Thor and Smith[2] analyzed freshly harvested pecan nuts at intervals from blossoming to maturity and found that most of the constituents of the kernel continued to increase until the nuts were fully mature. Other workers[3][4][5] have reported the analysis of kernels of cured nuts, but no work has been reported to show the effects of curing on kernel composition. Therefore, it was deemed advisable to determine (1) the effect of curing on the composition of nuts; (2) the effects of different curing temperatures on the composition of nuts, particularly of the kernels; and (3) whether nuts harvested at different stages of maturity are affected similarly by the curing process. The results of such a study are reported in this paper.

MATERIALS AND METHODS

Pecan (*Carya illinoensis* K. Koch; syn. *c. pecan* (Marsh.) Engl. and Graebn.) nuts of two standard varieties, Western and Success, growing in the orchard of the United States Department of Agriculture Pecan Field Station, Brownwood, Tex., were selected on the trees for uniformity of size and maturity and tagged on October 1, 1940. Some of these nuts were harvested at each of three later dates for curing and analysis. The first harvest of each variety was made when the nuts were fairly mature. At that time the shucks were green and had just begun to part at the sutures but had not opened enough for the nuts to begin drying. The harvesting of nuts at this stage of development is generally considered an early harvest. The first harvest was made on October 4 for the Western variety and on October 15 for the Success. The second harvest was made about 1 week after the first and is considered a midseason harvest. At this stage the shucks were green except around the sutures where they had opened. At these edges the shuck tissues had begun to turn brown and dry out. The shucks were still attached to the nuts, although they had opened up sufficiently to allow some drying of the exposed nut shells. The second harvest was

[1] Received for publication December 19, 1942.
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made on October 11 for the Western variety and on October 23 for the Success.

The third harvest was made when the nuts were fully tree-ripened and almost ready to drop-out of the shuck. In picking the nuts at this stage it was difficult to prevent separation of the shucks from the nuts. The Western nuts in this stage were harvested October 24; those of the Success were harvested November 14. The Success nuts in this late harvest were more mature than those of the Western variety harvested on October 24.

At each harvest date 450 nuts were collected and divided at random into 6 lots. Any poorly filled or defective nuts were discarded, and 67 nuts were finally used in each lot for curing and for sampling for analysis.

The six different lots for each harvest date and for each variety were treated as follows:

Lot 1. Separated into shucks, shells, and kernels and sampled immediately.
Lot 2. Shucks removed from nuts, and both cured at room temperature.
Lot 3. Nuts cured at room temperature without removing the shucks.
Lot 4. Shucks removed and nuts cured in refrigerator at $0°$ to $2°$ C.
Lot 5. Shucks removed and nuts cured in an air oven at $35°$ C.
Lot 6. Shucks removed and nuts cured in an air oven at $50°$ C.

Each lot of nuts subjected to curing was weighed at frequent intervals and was considered to be cured upon reaching approximately constant weight. After being cured, lots 2 and 3 were separated into shucks, shells, and kernels, and samples from each tissue were taken for analysis. The nuts of other lots were separated into shells and kernels which were sampled for analysis. The procedure was the same for nuts of each variety and for each harvest date, except that, in the case of the late-harvested nuts of lot 2, only the shells and kernels were analyzed.

The kernels were analyzed for reducing sugars; total sugars; acid-hydrolyzable polysaccharides; organic nitrogen; either extract, or oil; and dry matter. The shucks and the shells were analyzed for all these constituents except ether extract. Thor and Smith[6] found that freshly harvested shells contained no appreciable amount of hexose sugars or oil and that shucks contained no oil, but it was not known whether sugars would develop in the shells during the curing process.

The methods of sampling and analysis were the same as those used by Thor and Smith,[6] except that sugar analyses were made according to the method of Schaffer and Hartmann.[7]

The analytical data were calculated as milligrams or grams of constituents per kernel, shuck, or shell, based on the average dry weight of these tissues per nut on the date of sampling. By this method the actual changes in nut composition are apparent regardless of the percentage composition.

[6] See footnote 2.
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EFFECTS OF DATE OF HARVEST AND CURING ON VARIOUS CONSTITUENTS

The analytical data are summarized in tables 1 and 2. The amounts of reducing sugars in kernels of both varieties were extremely small, as shown in the tables, and are not discussed because the differences are within the limits of experimental error in sampling and analysis. Only traces of reducing and total sugars were present in the shells, and these data are omitted from the tables.

TABLE 1.—*Constituents per kernel, shuck, and shell of Western pecan nuts harvested on different dates and cured under different conditions*

Harvest date and lot	Curing temperature and conditions	Part of nut	Reducing sugars	Total sugars	Acid-hydrolyzable polysaccharides	Organic nitrogen	Ether extract	Dry matter
			Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Grams*	*Grams*
October 4, 1941:								
Lot 1	Sampled at harvest	Kernel	9.0	27.4	200.0	70.7	2.68	4.09
		Shuck	299.7	393.0	305.8	22.0		2.91
		Shell			557.8	6.9		2.74
Lot 2	Room temperature, shucks removed.	Kernel	10.0	86.0	144.3	70.6	2.92	3.88
		Shuck	86.8	136.6	356.2	21.0		2.44
		Shell			568.0	6.1		2.71
Lot 3	Room temperature, shucks attached.	Kernel	4.0	130.4	142.8	68.1	2.84	3.85
		Shuck	63.9	88.3	387.0	21.3		2.44
		Shell			497.8	5.6		2.75
Lot 4	0°-2° C., shucks removed.	Kernel	4.6	59.9	154.9	71.2	3.02	4.12
		Shell			538.7	6.6		2.79
Lot 5	35° C., shucks removed.	Kernel	8.7	99.0	149.0	71.3	3.06	4.10
		Shell			597.1	6.6		2.90
Lot 6	50° C., shucks removed.	Kernel	8.4	57.5	129.2	67.8	3.04	3.99
		Shell			542.6	7.1		2.49
October 11, 1941:								
Lot 1	Sampled at harvest	Kernel	11.5	49.9	223.6	75.0	3.31	4.49
		Shuck	333.0	487.3	310.0	23.1		2.84
		Shell			530.4	7.6		2.78
Lot 2	Room temperature, shucks removed.	Kernel	12.5	99.8	168.2	71.4	3.32	4.38
		Shuck	134.0	240.3	410.3	21.3		2.75
		Shell			508.3	7.4		2.89
Lot 3	Room temperature, shucks attached.	Kernel	12.7	105.0	172.6	72.8	3.33	4.36
		Shuck	95.0	176.0	388.0	20.8		2.61
		Shell			531.0	7.1		2.74
Lot 4	0°-2° C., shucks removed.	Kernel	10.7	70.5	146.1	72.8	3.10	4.07
		Shell			472.7	5.6		2.55
Lot 5	35° C., shucks removed.	Kernel	4.8	81.7	154.2	70.8	3.36	4.32
		Shell			546.9	6.5		2.75
Lot 6	50° C., shucks removed.	Kernel	7.2	73.2	170.6	70.4	3.42	4.42
		Shell			605.9	8.1		2.92
October 24, 1941:								
Lot 1	Sampled at harvest	Kernel	10.5	106.2	212.8	71.4	3.30	4.30
		Shuck	313.0	409.0	319.6	22.7		2.96
		Shell			544.4	8.1		2.87
Lot 2	Room temperature, shucks removed.	Kernel	13.0	113.0	222.9	75.8	3.45	4.54
		Shell			581.3	7.5		2.83
Lot 3	Room temperature, shucks attached.	Kernel	2.9	111.6	197.5	64.7	3.36	4.43
		Shuck	59.1	77.6	395.7	21.1		2.57
		Shell			533.9	7.8		2.80
Lot 4	0°-2° C., shucks removed.	Kernel	6.7	91.0	204.3	81.6	3.37	4.51
		Shell			509.5	6.4		2.70
Lot 5	35° C., shucks removed.	Kernel	7.6	108.1	198.8	81.6	3.44	4.56
		Shell			593.6	7.8		2.91
Lot 6	50° C., shucks removed.	Kernel	11.2	103.6	154.3	75.5	3.47	4.58
		Shell			552.0	8.5		2.91

TABLE 2.—*Constituents per kernel, shuck, and shell of Success pecan nuts harvested on different dates and cured under different conditions*

Harvest date and lot	Curing temperature and conditions	Part of nut	Reducing sugars	Total sugars	Acid-hydrolyzable polysaccharides	Organic nitrogen	Ether extract	Dry matter
			Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Grams*	*Grams*
Oct. 15, 1941:								
Lot 1	Sampled at harvest	Kernel	14.6	50.4	342.5	80.9	3.64	5.06
		Shuck	278.0	423.2	279.3	21.0	------	2.66
		Shell	----	----	809.9	10.7	------	3.99
Lot 2	Room temperature, shucks removed.	Kernel	10.7	155.0	163.2	82.5	3.59	4.80
		Shuck	45.0	60.9	364.5	21.0	------	2.25
		Shell	----	----	784.7	9.9	------	4.03
Lot 3	Room temperature, shucks attached.	Kernel	8.5	191.7	170.6	81.3	3.78	4.99
		Shuck	20.3	32.5	372.0	21.0	------	2.14
		Shell	----	----	664.0	9.8	------	3.76
Lot 4	0°–2° C., shucks removed.	Kernel	7.6	109.6	217.1	87.1	3.77	4.98
		Shell	----	----	771.2	11.1	------	4.14
Lot 5	35° C., shucks removed.	Kernel	11.5	114.8	170.3	82.4	3.61	4.88
		Shell	----	----	737.6	11.0	------	4.08
Lot 6	50° C., shucks removed.	Kernel	14.7	108.0	176.6	82.7	3.59	4.84
		Shell	----	----	884.4	13.1	------	4.07
Oct. 23, 1941:								
Lot 1	Sampled at harvest	Kernel	12.1	65.1	291.5	86.1	3.89	5.35
		Shuck	294.0	432.4	287.3	22.0	------	2.76
		Shell	----	----	912.7	11.7	------	4.16
Lot 2	Room temperature, shucks removed.	Kernel	9.3	149.2	202.6	101.8	4.05	5.39
		Shuck	48.1	84.7	393.8	24.0	------	2.36
		Shell	----	----	821.8	11.3	------	4.44
Lot 3	Room temperature, shucks attached.	Kernel	6.2	176.1	222.0	87.9	3.90	5.20
		Shuck	26.5	33.0	371.4	22.0	------	2.19
		Shell	----	----	744.1	10.8	------	3.90
Lot 4	0°–2° C., shucks removed.	Kernel	4.6	104.3	204.3	96.9	3.94	5.24
		Shell	----	----	713.4	10.8	------	3.92
Lot 5	35° C., shucks removed.	Kernel	10.9	137.8	180.7	95.0	3.95	5.25
		Shell	----	----	786.0	12.0	------	4.17
Lot 6	50° C., shucks removed.	Kernel	12.9	118.3	223.6	81.6	3.90	5.20
		Shell	----	----	864.4	12.1	------	4.16
Nov. 14, 1941:								
Lot 1	Sampled at harvest	Kernel	16.2	167.4	227.7	97.4	3.90	5.41
		Shuck	202.5	254.6	421.3	26.0	------	2.58
		Shell	----	----	846.4	11.3	------	4.26
Lot 2	Room temperature, shucks removed.	Kernel	14.5	164.6	218.9	86.8	3.91	5.20
		Shell	----	----	776.5	12.2	------	3.90
Lot 3	Room temperature, shucks attached.	Kernel	4.1	155.7	218.9	89.4	3.88	5.14
		Shuck	88.4	102.7	339.6	23.9	------	2.21
		Shell	----	----	730.7	9.7	------	3.85
Lot 4	0°–2° C., shucks removed.	Kernel	8.5	149.7	220.6	90.1	3.83	5.12
		Shell	----	----	733.2	10.3	------	3.89
Lot 5	35° C., shucks removed.	Kernel	42.0	163.5	208.4	93.4	3.82	5.16
		Shell	----	----	874.7	12.4	------	4.23
Lot 6	50° C., shucks removed.	Kernel	21.9	131.2	220.4	92.0	3.91	5.26
		Shell	----	----	851.9	11.6	------	4.17

REDUCING SUGARS

In shucks of freshly harvested nuts the amount of reducing sugars was relatively large. It increased slightly in the Western variety with the later harvest dates. In shucks of the Success variety the amount of reducing sugars increased slightly between the first and second harvest dates, but it decreased considerably between the second and third dates of harvest.

In the shucks of both varieties the reducing sugars decreased greatly during the curing period at room temperature, but in shucks of the Success variety harvested early and at midseason the decrease was greater than in those of the Western variety at comparable harvest dates. Also there was a greater decrease of reducing sugars in shucks of both varieties attached to the nuts during the curing process than in those that had been detached.

In shucks of late-harvested Success nuts the loss of reducing sugars during the curing process was less than in those of nuts harvested earlier.

TOTAL SUGARS

In kernels of both varieties total sugars had increased at each successive harvest date, there being nearly four times as much sugar per kernel in Western nuts harvested October 24 as in those harvested October 4, and more than three times as much in Success kernels harvested November 14 as in those harvested October 15. The sugars in the kernel were almost all nonreducing.

There was a great increase in total sugars during the curing period in the kernels of nuts of both varieties harvested early and at mid-season. In Western kernels harvested late (October 24) there was a slight increase in total sugars under all curing temperatures used except 0° to 2° and 50° C. In Success kernels harvested late (November 14) total sugars decreased somewhat during the curing process, the greatest decrease occurring in kernels cured at 50°.

The greatest increase of total sugars in kernels of both varieties was found in nuts of the first (early) and second (midseason) harvest dates that were cured at room temperature with the shucks attached. The least increase was in kernels of nuts cured at 0° to 2° and 50° C.

In the shucks, in contrast to the kernels, the greater part of the total sugars is reducing sugars, and as would be expected the changes in total sugars were parallel to those in the reducing sugars.

ACID-HYDROLYZABLE POLYSACCHARIDES

The acid-hydrolyzable polysaccharides of the kernels decreased considerably during the curing period in both varieties of nuts from the early and midseason harvests. There was only a slight decrease in this constituent during curing of Success kernels of the late harvest, but in late-harvested Western nuts it decreased considerably in the kernels cured at 50° C.

There was a slight decrease in acid-hydrolyzable polysaccharides of shells from early to late harvest in the Western variety. In shells of the Success variety there was a slight increase toward the later harvest dates, but there was no consistent change in this constituent in shells of either variety during curing. The acid-hydrolyzable polysaccharide content per shell of Success was much higher than that of Western, owing mainly to the larger size of the Success shells.

In shucks of both varieties the acid-hydrolyzable polysaccharide content was relatively high. It increased slightly in Western and markedly in Success during the ripening of the nuts on the trees and increased considerably when the shucks were cured at room temperature except in those of late-harvested Success nuts.

ORGANIC NITROGEN

There was no consistent change in the organic nitrogen content of kernels in either the Western or the Success variety during the curing process. Nitrogen increased appreciably in Success kernels with the later dates of harvest, but not in Western kernels.

The organic nitrogen content of the shucks was low in both varieties harvested early and at midseason and both contained almost the same amount per shuck, although the Western shucks contained more dry matter. There was not much change in this constituent in shucks during the curing process, but the shucks of the Success variety harvested late in the season (November 14) contained considerably more nitrogen than those harvested earlier.

The shells contained very small amounts of organic nitrogen, and there was no consistent change during the curing of the nuts. The higher amounts of organic nitrogen per shell in the Success variety were due largely to the fact that the Success shells were larger than those of the Western.

ETHER EXTRACT, OR OIL

In both varieties the amount of ether extract, or oil, per kernel increased from the early to the midseason harvest dates, after which it remained almost constant. In the Western variety there was an appreciable increase in the amount of ether extract per kernel during the curing process in nuts harvested early (October 4), but no appreciable change in this constituent in kernels of nuts harvested at the two later dates. There was no appreciable change in the amount of ether extract of Success kernels under any of the curing conditions. The amount of ether extract per kernel was greater in the Success variety than in the Western, because the Success kernels were larger.

DRY MATTER

There was an increase in the average amount of dry matter of kernels of nuts from the early to the midseason harvest dates, probably due largely to increases in oil content. The average increase in dry matter per kernel in the Western variety from October 4 (early harvest) to October 11 (midseason harvest) was 8.4 percent, or slightly more than 1 percent each day, whereas the average increase from October 11 to 24 was 3.4 percent. In the Success variety the average increase in dry matter was 7 percent from October 15 to 23, after which there was no further increase. These averages are based on the dry-matter content of all nuts in the six lots at each harvest and include the cured lots as well as those sampled immediately after harvest. In both varieties the increases are statistically highly significant.

The dry matter was slightly lower in kernels of most of the lots of both varieties after curing.

The amount of dry matter per shuck was greater at each harvest date in the Western variety than in the Success. In the shucks of both varieties the dry matter decreased slightly during curing at room temperature. In shells the dry-matter differences probably can be accounted for by variations in the original weight and size of the shells.

EFFECTS OF CURING TEMPERATURE ON FLAVOR, COLOR, AND TEXTURE OF KERNELS

The kernels of nuts cured at 50° C. were very brittle, and the flavor was similar to that of partly roasted kernels. The shells were

also very brittle. The color of the kernels was lighter than that of those cured at room temperature or at 35°. The moisture in the kernels averaged slightly more than 1 percent, which was approximately one-third of that of kernels cured at room temperature. These facts apply to all nuts cured at 50° regardless of the date of harvest.

The kernels of nuts cured at 0° to 2° C. were much lighter in color than those cured at 50°, but they lacked sweetness and appeared to be tough and flabby in texture. These effects were less pronounced in nuts of the later harvest dates than in those harvested early. The moisture content was only a little higher than that of kernels cured at room temperature.

Kernels of nuts cured at room temperature were normal for the variety in flavor, color, and texture, whereas those from nuts cured at 35° C. were normal in color and flavor but were somewhat brittle.

DISCUSSION

Certain interrelations may be noted in the changes occurring in the tissues of pecan nuts under the experimental conditions described.

SUGARS AND ACID-HYDROLYZABLE POLYSACCHARIDES

During the curing period at room temperature, the kernels of nuts of the early and midseason harvest dates with shucks attached increased more in total sugars (consisting chiefly of nonreducing sugars) than did the kernels of nuts with the shucks removed. This difference may have resulted from the translocation of some of the reducing sugars lost from the shucks during the same period and their transformation to nonreducing sugars in the kernels. However, a considerable part of the increase of total sugars in the kernels probably resulted from the conversion of other substances into sugars, since the kernels of nuts with the shucks removed also showed a considerable increase in total sugars under similar curing conditions.

The decrease in the acid-hydrolyzable polysaccharides of the kernels was sufficient to account for the increase in total sugars during curing, but the increase in sugars was not consistently proportional to the decrease in acid-hydrolyzable polysaccharides. Neither of these constituents changed materially during the curing process in nuts of the late harvest, except in those cured at 50° C.; and in the Success variety the acid-hydrolyzable polysaccharides in the kernels of freshly harvested nuts decreased with the later dates of harvest, while total sugars increased during the same period.

The relatively small increases of total sugars in kernels of nuts during curing at 0° to 2° and 50° C. indicate that these extremes of temperature were less suitable for sugar formation than room temperature.

In nuts cured with the shucks attached, the decrease in the reducing sugars of the shucks was greater than the increase of total sugars in the kernels. Moreover, none of the reducing sugars from detached shucks could have been translocated to kernels, yet these shucks lost a considerable amount of reducing sugars. It is possible that part of the reducing sugars was transformed into acid-hydrolyzable polysaccharides, which generally increased appreciably in the shucks during the curing period.

The slight changes in the acid-hydrolyzable polysaccharides of the shells of both varieties tend to confirm the finding of Thor and Smith [8] that shell formation is practically complete in the pecan nut before much development of the kernel takes place.

OTHER CONSTITUENTS

There were changes in other constituents of the nut tissues from harvest to harvest and during the curing process under various conditions. That nitrogen increased appreciably in Success kernels with the later dates of harvest, but not in Western kernels, may indicate that the proteins are later in forming in the Success than in the Western variety, since most of the organic nitrogen in pecan kernels is contained in the proteins.

Increases in dry matter of kernels from early to midseason harvest dates were probably due largely to increases in oil content; in both varieties these increases were statistically highly significant. The loss of dry matter in the kernels during curing may have been due to loss of carbon from respiration and to slight variations in the original size of the kernels.

PALATABILITY

The effects of the highest and the lowest curing temperatures (0° to 2° and 50° C.) on the palatability of the kernels were distinctly unfavorable. Only kernels cured at room temperature were normal for the variety in flavor, color, and texture, whereas kernels of nuts cured at 35° were normal in flavor and color but were slightly brittle.

SUMMARY AND CONCLUSIONS

A study has been made of the effects of harvest dates and curing on the composition of pecan nuts of two varieties, Western and Success.

Some important changes in composition occurred in kernels and shucks of nuts from early to midseason or late harvest dates and during the curing periods. The amount of total sugars increased greatly in kernels as the date of harvest advanced; it also increased considerably during the curing periods in kernels of nuts of the early and midseason harvests, the greatest increase occurring in kernels of nuts cured at room temperature with the shucks attached. The least increase of total sugars during curing occurred in kernels of nuts cured at 0° to 2° and at 50° C. Acid-hydrolyzable polysaccharides decreased in the kernels during curing, and some of these substances may have been converted into sugars. The shucks lost a considerable amount of reducing sugars during the curing period, while an increase in acid-hydrolyzable polysaccharides occurred at the same time. This indicates that at least a part of the reducing sugars was converted into acid-hydrolyzable polysaccharides.

Acid-hydrolyzable polysaccharides decreased slightly in the shells of the Western variety with the later dates of harvest, but the reverse was true in shells of Success nuts. There was no consistent change in this constituent during the curing process in shells of either variety.

[8] Thor, C. J. B., and Smith, C. L. A physiological study of the prefilling period of fruit development in the pecan. Jour. Agr. Res. 58: 905–910, illus. 1939.

There was no appreciable change in organic nitrogen of the tissues during curing, or for different harvest dates, except that it increased in Success kernels with the later dates of harvest. This may indicate that proteins continue to be formed at later stages of development in Success kernels than in Western kernels, since most of the organic nitrogen of the kernels is contained in the proteins.

Ether extract, or oil, increased in kernels of both varieties from the early to the midseason harvest dates, after which it remained almost constant. There was a slight increase of oil in early harvested Western kernels during the curing period, but the oil content remained almost constant in all others.

There was a statistically significant average increase in dry matter of kernels of nuts of both varieties harvested at midseason as compared with those harvested early, probably due largely to increases in the oil content. These average increases amounted to 8.4 percent and 7 percent in the Western and Success varieties, respectively.

Kernels of nuts cured at 50° C. were very brittle, and the flavor was impaired in that they tasted somewhat like partly roasted kernels. This was true of all nuts cured at 50°, regardless of date of harvest. Kernels of nuts cured at 0° to 2° lacked sweetness, the texture was flabby and tough, and the color was lighter than that of kernels of nuts cured at the higher temperatures; but these effects were less pronounced in nuts of the later harvest dates than in those harvested early. Kernels of nuts cured at room temperature appeared to be normal in flavor, color, and texture regardless of the date of harvest, while kernels of nuts cured at 35° were normal in flavor and color but were somewhat brittle.

A DISEASE OF GLOXINIA CAUSED BY PHYTOPHTHORA CRYPTOGEA[1]

By John T. Middleton, *assistant plant pathologist, California Agricultural Experiment Station;* C. M. Tucker, *Chairman, Department of Botany, Missouri Agricultural Experiment Station;* and C. M. Tompkins, *associate plant pathologist, California Agricultural Experiment Station.*

INTRODUCTION

Gloxinia (*Sinningia speciosa* Benth. and Hook.) is a highly prized floricultural plant, in California primarily grown in pots for the florist trade. Plants are grown either from seeds or corms (commercially termed "tubers"), usually under glass though occasionally under lath in protected coastal areas. The culture of gloxinia represents an appreciable capital investment.

During the summer of 1938 a disease caused by *Phytophthora cryptogea* Pethyb. and Laff. affecting the corm, stem, and leaves of the plant was observed on potted and bench-grown plants in several large commercial glasshouses in Capitola and San Francisco, Calif. The disease was of economic importance, causing a considerable loss of plants.

It is the purpose of this paper to discuss the symptoms of the disease, the causal organism, and its host range.

REVIEW OF LITERATURE

Few reports appear in the literature of a gloxinia disease which resembles the condition found by the authors. With the exception of a preliminary report by two of the authors (8)[2] the reports that do exist are all from Europe, none originating on this continent. Apparently the first record of a leaf and stem rot of gloxinia caused by a species of *Phytophthora* is a report from the Netherlands by Van Poeteren (10) in 1926. Landgraf (4) briefly describes a corm, stem, and leaf rot in Germany in 1932; no specific organism is reported to be responsible, *Botrytis*, *Moniliopsis*, *Phytophthora*, and *Pythium* all being associated with the disease. Mehlisch (6) in 1935 wrote a short description of a soft rot of gloxinia to which he applied the name "Falscher Mehltau," or false mildew; the causal organism was referred to as *Phytophthora speciosa*, but was not described. In 1936 (2) *P. parasitica* Dastur was said to be the agent responsible for a stem and leaf rot of gloxinia in England and Wales. Pape (9) in 1937 reported a stem and leaf rot in Germany and made several suggestions for the control of the disease as it occurs in the glasshouse; an unidentified *Phytophthora* sp. was said to be associated with the disease. Williams et al. (17) report that *P. cactorum* (Leb. and Cohn) Schroet., isolated from *Erica hiemalis* Hort. in England, was capable of infecting gloxinia stems upon inoculation. The most recent contribution is that of Schmidt (13) who reiterates the remark of Mehlisch that gloxinia mildew is caused by *P. speciosa*.

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Some of the disease symptoms on gloxinia due to *Phytophthora* may resemble those arising from other causes. Meurs (*7*) states that *Pythium debaryanum* Hesse is responsible for a root rot of gloxinia in the Netherlands. Van Poeteren (*11, 12*) reports soft, black, sunken lesions on gloxinia corms received in the Netherlands from Belgium and said to be due to *Cylindrocarpon radicicola* Wr. Marchal (*5*) records *Thielavia basicola* (Berk. and Br.) Zopf from gloxinia corms in Belgium, stating that the fungus caused superficial, black encrustations on affected corms. *Thielaviopsis basicola* (Berk.) Ferraris has been reported from corms in England and Wales (*1*).

SYMPTOMS OF THE DISEASE

The disease affects the corm, stems, and leaves of the plant. The symptoms of the disease are identical on all varieties of gloxinia that have been found infected. In naturally infected plants the leaves are somewhat discolored, water-soaked, dark brown, and quite flaccid (fig. 1). Within 2 to 7 days, under favorable conditions for the de-
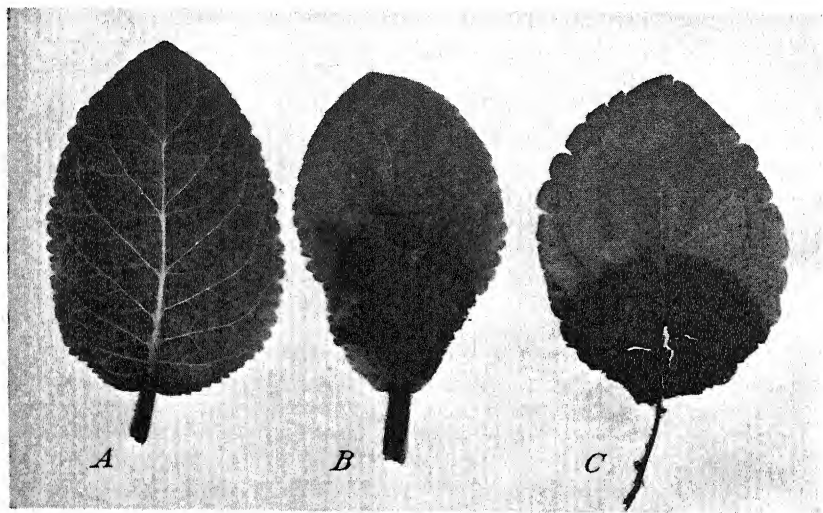
FIGURE 1.—Leaf rot of gloxinia: *A*, Noninoculated control; *B*, *C*, symptoms produced on leaves of gloxinia by *Phytophthora cryptogea* 12 days after inoculation in the glasshouse.

velopment of the disease, infection may progress from the lamina of the leaf to the petiole, causing the death and collapse of the leaf. In badly diseased plants the stems may be attacked; quite often stem infection is noted in the absence of leaf infections. Infected plants at first appear stunted and wilted (fig. 2), later they may become chlorotic, collapsing as infection spreads into the petioles (fig. 3). Diseased stems bear sunken, water-soaked lesions which may be rather narrow and vertically disposed, or may be quite large and encompass the stem.

Corm infection may be associated with stem or leaf infection or may occur independently. Infected corms exhibit soft, sunken, surface lesions. Severely diseased corms usually have dark-brown, soft,
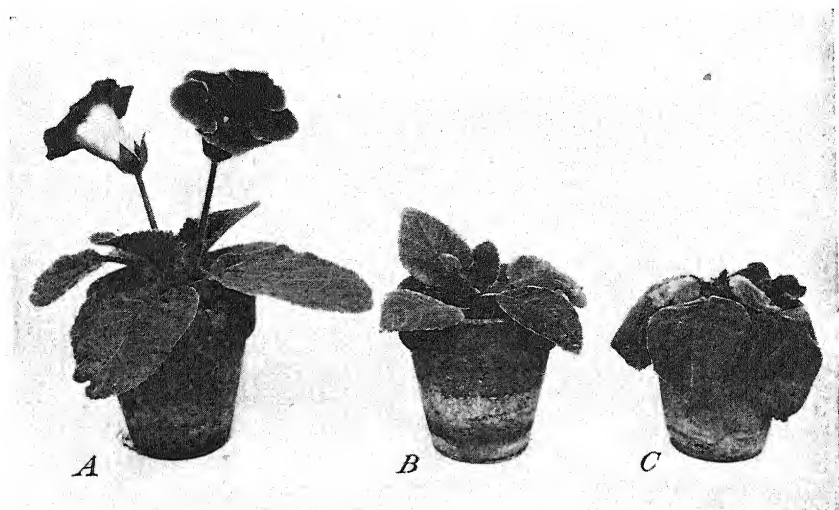
FIGURE 2.—Phytophthora disease of gloxinia: *A*, Noninoculated control; *B,* stunting, and *C*, wilting produced on gloxinia plants by *Phytophthora cryptogea* after inoculation in the glasshouse.
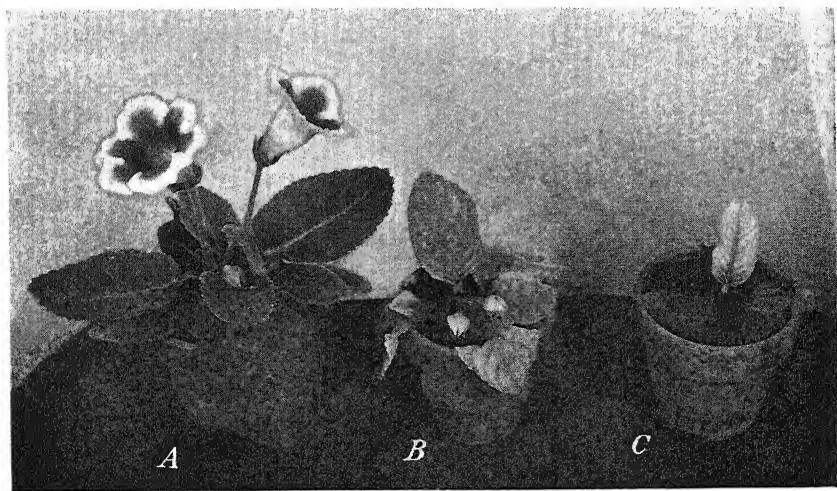
FIGURE 3.—Phytophthora disease of gloxinia: *A*, Noninoculated control; *B, C,* chlorosis and rot produced on gloxinia plants by *Phytophthora cryptogea* **after** inoculation in the glasshouse.

internal necrotic areas, 1 to 8 mm. in diameter, erratically disposed throughout the underground storage organ (fig. 4). These infected regions may or may not be directly associated with the more common surface lesions. The fibrous roots of diseased plants are often infected, particularly those roots that are associated with infected areas on the surface of the corm. Infected roots are darkly discolored and flaccid.

Recently, Ark and Tompkins (*3*) have described a boron-deficiency disease of glasshouse-grown gloxinia in which the leaf lamina shows a brownish-black discoloration and loss of turgidity, eventually causing the plants to die. This disease may sometimes be confused with the one discussed in this paper.
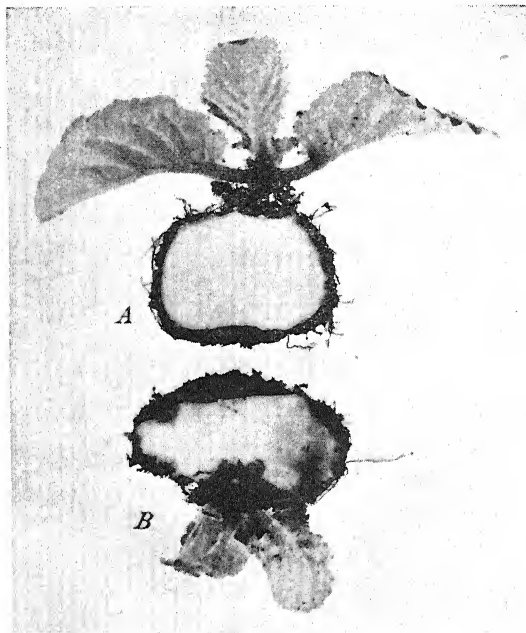
FIGURE 4.—Corm rot of gloxinia: *A*, Noninoculated control; *B*, symptoms produced on gloxinia corms by *Phytophthora cryptogea* 28 days after inoculation in the glasshouse.

THE CAUSAL FUNGUS

Isolations made from diseased leaves, stems, and corms on water or malt-extract agars have consistently yielded a fungus which has been identified as *Phytophthora cryptogea*.

MORPHOLOGY

Reproductive bodies rarely develop on the usual agar media; in old cultures on oatmeal agar, oögonia with a mean diameter of approximately 25μ were occasionally found. Each oögonium contained an oöspore, the mean diameter of which was approximately 22μ. The antheridia were of the persistent, amphigynous type. When hyphae

were transferred to distilled water or nonnutrient solutions, sporangia developed in a few days. They were nonpapillate, ovoid, obpyriform or elongated, straight or slightly curved, with a mean size of approximately 35μ long and 20μ wide. Small spherical to irregular vesicular hyphal swellings developed in large numbers; they were hyaline, thin-walled and usually disposed in dense clusters.

TEMPERATURE RELATIONS

The relation of temperature to growth of the mycelium was determined for four isolates of *Phytophthora cryptogea* from leaves, stem, and corm. The culture tubes used and the procedure followed in this study are those previously described by Tompkins and Gardner (*14*). Each tube used is provided with a glass dam at the open end. Thirteen milliliters of Difco corn-meal agar (pH 6.0) are added to each tube and allowed to cool to a uniform depth with the vessel in a horizontal position; the dam prevents the escape of the melted agar.

The tubes were planted near the dam at the open end with a small square of potato-dextrose agar containing the mycelium of the fungus cut from 5-day-old Petri-dish cultures. Inoculated tubes were left at room temperature for 36 hours, the extent of growth at the close of the period being indicated by a wax pencil mark on the tube; subsequent measurements were made from this point.

Three tubes of each isolate were placed in a horizontal position in controlled temperature chambers at intervals of 3°, from 1° to 34° C. The cultures were incubated for a 96-hour period. The amount of growth in millimeters for the 96-hour period at the various temperatures is presented in table 1.

TABLE 1.—*Mycelial growth of Phytophthora cryptogea on Difco corn-meal agar at various temperatures*

P. cryptogea from corm, leaf, and stem	Growth in millimeters over 96-hour period											
	1° C.	4° C.	7° C.	10° C.	13° C.	16° C.	19° C.	22° C.	25° C.	28° C.	31° C.	34° C.
Corm	6	9	12	18	26	36	46	55	58	47	30	0
Leaf	5	8	13	18	25	36	48	56	58	43	21	0
Do	6	10	13	16	24	35	45	53	55	45	25	0
Stem	7	10	13	17	25	36	47	55	57	46	27	0

The minimum temperature for mycelial growth is below 1° C., the optimum between 22° and 25°, and the maximum between 31° and 34°. These values are in conformity with those given by Tucker (*16*).

PATHOGENICITY

All isolates of the fungus from gloxinia proved pathogenic to healthy gloxinia plants grown from seeds and corms. Inoculum was prepared by growing the fungus on sterilized cracked wheat in 8-inch test tubes. When ready for use, this was added to autoclaved soil contained in 6-inch pots in such a manner as to avoid injuring the root system of the young plants. Sterile cracked wheat was added to pots serving as controls. All pots were heavily watered each day in order to keep the soil very moist. The incubation period ranged from 14 to 28 days

for seedling plants, but all infected plants died within 2 to 6 days after the foliage commenced to wilt. The incubation period for plants from corms was somewhat longer, depending upon the size of the corm planted; the range was usually from 18 to 35 days. Of 435 plants inoculated, none escaped infection, while all the control plants remained healthy. The fungus was reisolated from a representative sample of plants and proved to be identical with the original isolates; when tested in parallel series, the reisolates again proved pathogenic.

Symptoms on artificially infected plants were identical with those on naturally infected plants. Contrary to the report of Pape (*9*) that the blue-flowered varieties of gloxinia are most susceptible to infection by *Phytophthora* sp. than the white varieties, no difference in susceptibility was found in blue, white, red, or variegated varieties when inoculated with *P. cryptogea*. However, this does not exclude the possibility of the existence of varieties resistant to this disease, as only a relatively few varieties were tested.

EXPERIMENTAL HOST RANGE

Various hosts were inoculated by the technique described for the pathogenicity tests. Reisolations were made from all diseased specimens; the reisolate of the fungus from a particular host was then tested by inoculation into healthy plants of that host.

A number of species of the Gesneriaceae were first tested. Approximately 40 plants of each species were inoculated with *Phytophthora cryptogea* from gloxinia. Plants of *Achimines cardinalis* A. Dietr., *A. grandiflora* DC., *A. longiflora* DC., *Aeschynanthus lobbiana* Hook., *A. speciosus* Hook., *Alloplectus schlimii* Planch. and Lind., *Episcia cupreata* Hanst., *Gesneria cardinalis* Lehm., *Isoloma amabile* Mott., *I. hirsutum* Regel, *Naegelia cinnabarina* Lind., *N. multiflora* Hook., *N. zebrina* Regel, *Saintpaulia ionantha* Wendl., *Sinningia speciosa*, and *Streptocarpus kewensis* Hort. proved to be suscepttible.

A résumé of natural and artificial infection of certain plants by *Phytophthora cryptogea* as reported by various investigators has been given by Tompkins and Tucker (*15*), who have presented also several new host records for this fungus. No members of the Gesneriaceae are indicated. The fungus has been isolated and identified by the writers more recently from *Prunus amygdalus* Batsch (almond) and *Cotoneaster lacteus* W. W. Smith. Cultures of the species isolated from *Dianthus caryophyllus* L. (carnation) and *Ceanothus prostratus* Benth. were received from Kenneth F. Baker.

Isolations of *Phytophthora cryptogea* from *Calceolaria crenatiflora* Cav. (slipperwort), *Callistephus chinensis* Nees (China-aster), *Celosia argentea* L. var. *cristata* (L.) O. Ktze. (cockscomb), *Godetia grandiflora* Lindl. (godetia), *Iris* sp., *Senecio cruentus* DC. (cineraria), gloxinia, and *Tagetes erecta* L. (African marigold) were used in inoculation experiments to determine the susceptibility of slipperwort, China-aster, cockscomb, *Gerbera jamesonii* Hook. var. *transvaalensis* Hort. (Transvaal daisy), godetia, *Matthiola incana* R. Br. var. *annua* Voss (annual stock), cineraria, and gloxinia to infection. The results are presented in Table 2.

Annual stock, China-aster, Transvaal daisy, and cineraria have previously been reported (*15*) as susceptible to *Phytophthora cryptogea*.

TABLE 2.—*Artificial inoculation of various plants with different isolates of Phytophthora cryptogea*

Host from which isolated	Plant inoculated [1]							
	Annual stock	China-aster	Ciner-aria	Cocks-comb	Gloxinia seed-ling	Gode-tia	Slip-perwort	Trans-vaal daisy
African marigold	+	−	+	+	+	−	−	+
China-aster	+	+	+	+	−	−	−	+
Cineraria	+	+	+	+	+	+	+	+
Cockscomb	+	−	+	+	+	−	+	+
Gloxinia:								
Corm	+	+	+	−	+	+	+	+
Stem	+	−	+	+	+	−	+	+
Godetia	+	−	+	−	−	−	+	+
Iris	+	−	+	−	−	−	−	+
Slipperwort	+	−	+	+	+	+	+	+

[1] + = Positive infection; − = no infection.

DISCUSSION

In the absence of any description of *Phytophthora speciosa*, which is supposedly a new species responsible for a rot of gloxinia in Europe, the epithet must be considered a nomen nudum. In England a disease similar to that caused by *P. speciosa* is said to be due to *P. parasitica*. The authors have recovered only *P. cryptogea* from diseased gloxinia; this is believed to be the first report of a *Phytophthora* disease of gloxinia in America, the first record of the occurrence of *P. cryptogea* on this host, and the first report of this species on the leaves of any host.

The causal fungus is able to infect not only gloxinia, but also a number of other species of the Gesneriaceae. The following are all new host records for *Phytophthora cryptogea: Achimines cardinalis, A. grandiflora, A. longiflora, Aeschynanthus lobbiana, A. speciosus, Alloplectus schlimii, Episcia cupreata, Gesneria cardinalis, Isoloma amabile, I. hirsutum, Naegelia cinnabarina, N. multiflora, N. zebrina, Saintpaulia ionantha,* and *Streptocarpus kewensis* Hort.

It is apparent from the results given in table 2 that annual stock, cineraria, and Transvaal daisy are more susceptible to attack by *Phytophthora cryptogea* than China-aster, cockscomb, gloxinia, godetia, and slipperwort. There is also some evidence that certain isolates of *P. cryptogea* may have a wider host range than others; the isolate from cineraria was able to infect annual stock, China-aster, cineraria, cockscomb, gloxinia, godetia, slipperwort, and Transvaal daisy, whereas the isolate from iris was able to infect only annual stock, cineraria, and Transvaal daisy, hosts which were shown to be susceptible to all nine isolates used.

Cineraria, cockscomb, and slipperwort are new hosts for *Phytophthora cryptogea* and so far as the writers are aware have not been previoulsy recorded as such in the literature.

SUMMARY

A corm, stem, and leaf disease of gloxinia is prevalent on the San Francisco Peninsula and in the vicinity of Capitola, Calif.

Symptoms of the disease consist of soft, sunken, surface lesions and

internal dark-brown, soft necrotic areas, 1 to 8 mm. in diameter. Diseased stems bear sunken, water-soaked lesions which may be rather narrow and vertically disposed, or may be quite large and encompass the stem. Infected leaves are water-soaked, dark brown, and flaccid.

The causal organism has been identified as *Phytophthora cryptogea* Pethyb. and Laff.

The minimum temperature for mycelial growth is below 1° C., the optimum between 22° and 25°, and the maximum between 31° and 34°.

Phytophthora cryptogea has been demonstrated to be pathogenic to 9 genera and 15 species of the Gesneriaceae in addition to gloxinia.

Cineraria, cockscomb, and slipperwort are newly reported hosts for *Phytophthora cryptogea*.

This is apparently the first record of *Phytophthora cryptogea* on gloxinia, and the first instance of this fungus on leaves of any host.

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RELATION BETWEEN HOT-WATER EXTRACTIVES AND DECAY RESISTANCE OF BLACK LOCUST WOOD [1]

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INTRODUCTION

The heartwood of black locust (*Robinia pseudoacacia* L.) shows considerable variation in durability. Within the commercial range of black locust, farmers commonly refer to at least two kinds of locust in respect to durability, viz, (1) "yellow" locust, characterized by a dark-yellow heartwood, which is reputed to last 50 to 100 years in contact with the soil, and (2) "white" locust, characterized by a gray heartwood, which is reputed to last only 10 to 30 years in contact with the soil. Less frequently, such terms as "green," "red," "gray," and "black" are used by farmers in describing color variations of heartwood that in their experience are associated with differences in durability.

Hirt (7)[4] investigated this reputed difference by laboratory culture tests of the wood of two selections of locust from Long Island, N. Y.: Shipmast locust, which is a "yellow" variety; and common locust, which is a "white" variety. One tree of each variety was tested. The study showed that the heartwood of the shipmast locust tree was more resistant to decay than the heartwood of the common locust tree.

Subsequently, heartwood from a large number of trees, representing several different varieties, was tested by the writers. The results, as yet unpublished, indicate definitely that there are significant differences between varieties in the durability of their wood. The tests show also that smaller but nevertheless substantial differences in wood durability exist between trees of different sizes and ages belonging to the same variety, as well as between different positions within the same tree.

These laboratory investigations and field observations indicate that locust strains of superior wood durability may be selected for large-scale planting to replace the unselected locust used at present, provided a simple, rapid method can be developed to test the wood durability of the many varieties under consideration. Studies by different investigators on several tree species having durable wood indicate that resistance of the heartwood to decay is commonly due in large measure to the presence of toxic extractives. It therefore appeared desirable to determine whether a simple extractive test could

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be used as a practical index of the durability of the wood of the various black locust selections.

Two separate experiments were conducted in the study reported here. The first experiment was a test with extractives from black locust heartwood to determine the effect of various concentrations of the same extractives on the growth rate of an appropriate decay fungus. The second experiment was a test of various black locust trees and strains to determine whether their relative resistance to decay may be ascertained by measuring either the concentration of extractives in the heartwood or the toxicity of the extractives to the decay fungus.

REVIEW OF LITERATURE

Working with 15 woods, including both British and North American species, Ernest (5) showed that many of them had water-soluble extractives that were in various degrees toxic to decay fungi. Hawley et al. (6) found that the cold-water and hot-water extractives from 10 native species of wood are toxic to the growth of a wood-destroying fungus. They concluded that the generally accepted relative resistance to decay of the woods tested could be largely explained by the toxicity of their water-soluble components. Black locust was one of the woods in which the presence of toxic extractives was demonstrated. A similar finding was reported by Sowder (12) and by Cartwright (4) for western red cedar (*Thuja plicata* D. Don), and chemists at the Forest Products Laboratory (unpublished data) have isolated several toxic extractives from the heartwood of that species. Moderately toxic water-soluble extractives have even been found in ponderosa pine (*Pinus ponderosa* Dougl.) (1), a species with comparatively low decay resistance.

The resistance to decay of various parts of the heartwood of virgin redwood (*Sequoia sempervirens* (Lambert) Endlicher), as shown in laboratory tests, was found by Sherrard and Kurth (10) to vary with the distribution of the water-soluble extractives. A similar relationship was noted in a limited preliminary study on two black locust trees by Hopp and Hirt.[5]

MATERIALS AND METHODS

SELECTION OF TREES

Eleven black locust trees, cut on Long Island, N. Y., were used in this study. Two of the trees belonged to the shipmast selection (8); four represented an undescribed variant designated as Flowerfield locust; and the remaining five were of a heterogeneous nature, grouped under the general name "common locust." As the shipmast and Flowerfield variants bear practically no seed, the individual trees of each of these two groups were probably closely related genetically. The common locust trees were seed-bearing types and came from separate stands; hence, several variants were probably represented. The trees were all approximately 10 inches in diameter at breast height.

PREPARATION OF TEST BLOCKS

Materials for testing were taken from 1-foot sections of the trunks at heights of 7 to 8 feet. From the heartwood of these sections were sawed, in radial order, groups of five small blocks, consisting of four

decay specimens [6] and one reference specimen for computing the initial oven-dry weights of the decay specimens (fig. 1). The test blocks were ½ by 2 by ¼ inches in the radial, tangential, and longitudinal directions of the wood, respectively. In addition to the small blocks, one larger block was taken at each radial position for the extractive analyses. The blocks were numbered consecutively according to their respective radial position, inward from the sapwood to the pith. Each position, which included the width of a test specimen and saw kerf, occupied approximately 0.65 inch on a radius.
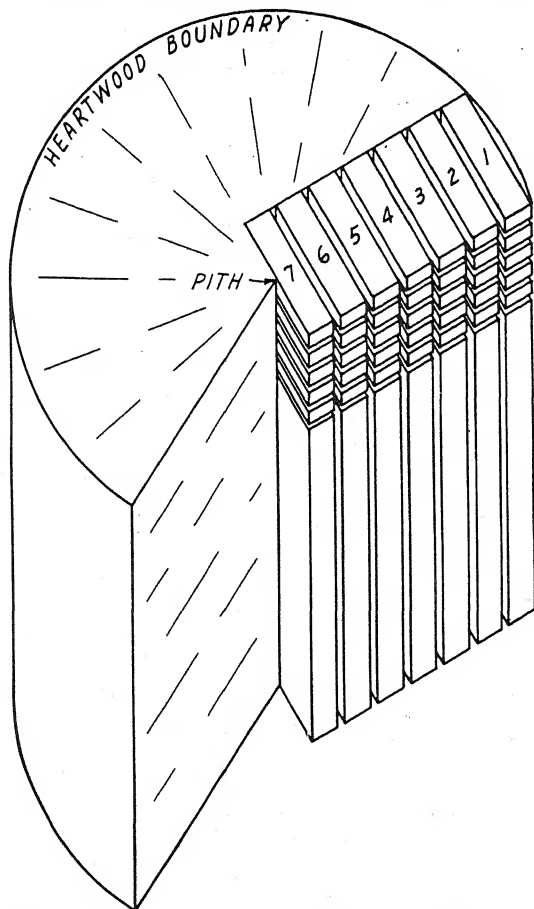
FIGURE 1.—Blocks for decay tests (small blocks) and for extractive comparisons (large blocks) in a tree section. All blocks in each vertical series were given the same serial number, beginning with the heartwood boundary as indicated.

The decay tests and the extractive analyses at any particular radial position were thus made on virtually replicate material, since the wood in each group of blocks came from essentially the same place in the same growth rings. Each radius was completely sampled except where suitable material, free from knots or locust-borer damage, was not available.

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[6] Only two of the four decay blocks were used in the present study; the other two were used for tests not considered here.

DECAY TESTS

The amount of decay produced in the blocks by the test fungus was measured by the percentage loss in the oven-dry weight of the wood. The blocks were first dried in a special room where the relative humidity was maintained at 30 percent and the temperature at 80° F. The blocks were then weighed and sterilized by heating in an autoclave for 20 minutes at 100° C. Following this, the blocks to be decayed were placed, under aseptic conditions, in 6-ounce wide-mouth bottles, of the French square type, having screw tops from which the paper lining had been removed. Each bottle contained 25 ml. of malt-agar medium (2.5 percent of Trommer's plain diastasic malt extract and 1.5 percent of Bacto-agar in distilled water), which had hardened while the bottles were in a horizontal position. The specimens were laid, one in a bottle, on a 3-mm. v-shaped glass rod resting on the hardened substrate, and the substrate was inoculated immediately with a pure culture of the test fungus. The cultures were then incubated for 4 months at a constant temperature of 80° F. and a relative humidity of 80 percent. At the end of the incubation period the blocks were wiped free of loose surface growth of the fungus, oven-dried at 105° C., and again weighed. The reference blocks, which had been stored in dry containers, were oven-dried and weighed at the same time. In determining weight losses, the initial oven-dry weights of the decayed blocks were computed from their initial air-dry weights and the weight losses on drying of the corresponding undecayed reference blocks.

DETERMINATION OF EXTRACTIVE CONTENT

For the extractive determinations, the large block from each radial position was reduced to sawdust and the proportion of oven-dry wood soluble in hot water was determined in accordance with the standard procedure followed at the Forest Products Laboratory (*3*). The initial oven-dry weight of the extractive samples was computed rather than determined directly, according to the procedure described for the decay specimens. The hot-water extractive rather than the cold-water extractive was measured because of indications in the work by Hopp and Hirt[7] that the former more closely paralleled decay resistance. Each extractive analysis was made in duplicate. The solutions of extractive material were carefully retained for the toxicity tests.

MEASUREMENT OF EXTRACTIVE TOXICITY

EXPERIMENT 1

The relative toxicity of the extractives was measured by the linear growth rate of the decay fungus on the standard culture medium (see Decay Tests) containing the extractives. For the first experiment, in which the toxic effect of different concentrations of the same extractives was to be determined, extracts from two samples were used. These samples were obtained from (1) radial position 1 of Flowerfield tree B46–2 and (2) radial position 1 of common tree A54–18, trees that differed markedly in decay resistance. The extractives were tested at seven different concentrations, as listed in the results.

[7] See footnote 5, p. 422.

Since the extract solution contained known amounts of extractive, the desired extractive concentrations in the culture media were obtained by diluting or by evaporating portions of the extract solutions, as required. The solutions were evaporated, in beakers, at 50° C., or diluted to a few milliliters less than the final volume, and the requisite quantities of malt extract and agar were finally added to each preparation. The mixtures were then heated in an autoclave for 20 minutes at 100° to liquefy the agar. Following this, enough hot distilled water was added to the preparations to give exactly the predetermined total amounts of medium required for the desired concentrations of extractives. The resultant hot medium was then mixed by blowing air into it through a pipette, and immediately thereafter was pipetted into special cotton-plugged test tubes, where it was first sterilized by autoclaving or steaming and then allowed to harden. Ten milliliters of medium was placed in each tube. To reduce evaporation losses and attendant changes in the composition of the medium, the tubes were capped with lead foil.

Because of the possibility that autoclaving might tend to alter the extractives more than a milder sterilization treatment, two types of sterilization were tried: (1) Heating for 1 hour at 100° C. on 3 successive days and (2) autoclaving for 15 minutes at 15 pounds, steam pressure. The latter type of sterilization was used throughout the second experiment.

The test tubes in which the growth-rate measurements were made were 2.5 cm. in diameter and 20 cm. long and were modified by a rounded indentation in the wall near the mouth (9). The medium was cooled with the tubes in a horizontal position, and the indentation formed a dam that prevented escape of the medium while it was still liquid. The agar strip thus formed provided a substrate about 15 cm. long and of uniform cross section throughout the length of the tube.

The medium was inoculated near the midpoint with a small piece of mycelial mat of the test fungus, and subsequent linear growth in both directions was measured from a mark made on the glass. The temperature in the incubation chamber was approximately 28° C., although some small variation was unavoidably produced by occasional higher outside temperatures. The total linear extension of both advancing margins of the fungus occurring between the fourth and the ninth day following inoculation was taken as the measure of rate of growth, 4 days being allowed for the fungus to become well established on the new substrate. To put the measurements for this experiment on the same time basis as those for the second experiment, the respective rates were computed to a 7-day period.

EXPERIMENT 2

In the second experiment, the extractive contents were determined for each radial position of all the trees dealt with, but the toxicity of the extractions was tested only on the Flowerfield and common locust trees, since these selections showed the greatest difference in decay resistance. The toxicity tests of samples from the Flowerfield trees were made in duplicate and of those from the common trees in quadruplicate. The mixtures of extractive and malt agar were prepared for these tests in each case by evaporating all the extractive solution

from a 2-gm. sample of sawdust to a volume of about 20 cc., then adding sufficient malt extract, agar, and distilled water to give a total volume of hot culture medium of 25 ml. The final concentrations of malt extract and agar were, as in the previous media preparations, 2.5 percent and 1.5 percent, respectively, and the concentrations of extractives in the media were between 0.25 and 1.0 percent, which, from the results of the first experiment, were judged to lie in a range most suitable for comparing toxicities as measured by the growth rate of the test fungus. The rest of the procedure in making the toxicity tests was the same as that followed in experiment 1. The total amount of linear extension of the fungus during the 7-day period from the fourth to the eleventh day after inoculation was used as the measure of growth rate.

For both the decay tests and the toxicity tests, a strain of *Fomes rimosus* (Berk.) Cke. was used that had been isolated from a decayed locust fence post.

RESULTS

RELATION BETWEEN THE CONCENTRATION AND TOXICITY OF BLACK LOCUST EXTRACTIVES (EXPERIMENT 1)

The objectives of this experiment were (1) to obtain definite verification of the toxic nature of black locust extractives, (2) to determine what concentrations of extractives would give a suitable range of growth rates for the toxicity tests of the second experiment, and (3) to determine which type of sterilization (autoclaving or steaming) should be used. In addition, quantitative data were obtained on the experimental error attached to such biological measurement of the toxicity of locust extractives.

The results of the experiment are presented graphically in figure 2. It is apparent that the tested concentrations of extractive lower than
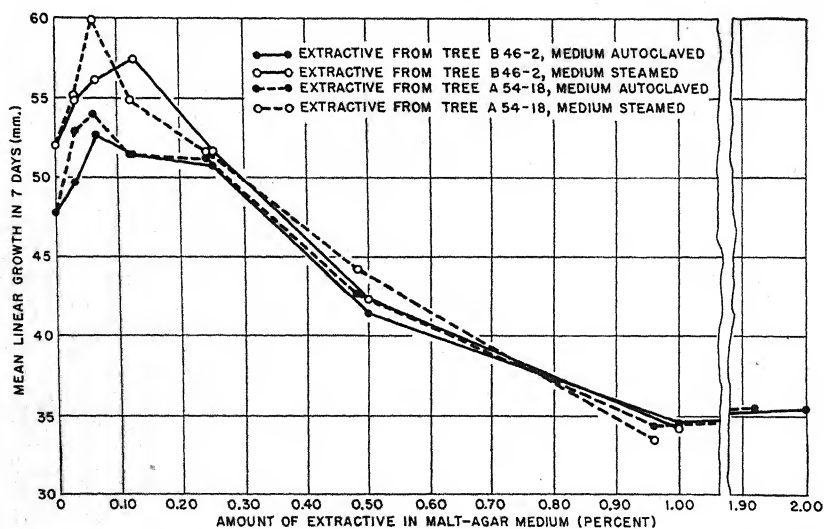
FIGURE 2.—Rate of growth of *Fomes rimosus* on malt-agar medium, autoclaved or steamed, containing various concentrations of extractives from radial position 1 in trees B46–2 and A54–18. (Experiment 1.)

about 0.25 to 0.30 percent stimulated in various degrees rather than retarded growth of the fungus. Such a phenomenon is not uncommon with other decay fungi and with other toxic chemicals in low concentrations (*2*). On the other hand, growth retardation is indicated for all concentrations above about 0.25 to 0.30 percent, and in increasing degree to a concentration of about 1 percent. At concentrations of about 2 percent the growth rate was nearly the same as at about 1 percent, the small difference probably being due to experimental error. This break in the trend was possibly a result of the medium being saturated with toxic ingredients of the extract solutions at concentrations above the 1-percent level. Such a conclusion is further suggested by the presence in increasing amounts of a brown and yellow precipitate in media containing extractives in concentrations of more than 0.24 percent.

The two methods of sterilization did not cause a significant difference in growth rate of *Fomes rimosus* when the extractive concentrations were large enough to cause retardation. When the extractive was absent from the medium or was at a low concentration, in the range of stimulation, steaming was associated with more rapid growth than autoclaving. Consequently, the toxicity tests in experiment 2 were made with concentrations of the extractives in the range of retardation and the media were autoclaved because of the simpler procedure required.

The average variation between results of duplicate cultures indicated that the smallest difference in concentration of extractive in malt agar that can be reliably ascertained by growth-rate measurements is about 0.1 percent.

RELATION OF DECAY RESISTANCE TO THE CONTENT AND TOXICITY OF EXTRACTIVES IN THE WOOD (EXPERIMENT 2)

The weight loss due to decay and the corresponding content and toxicity (in terms of fungus growth rate) of the extractives at each radial position in the trees tested are given in table 1.

The first thing to consider is what variations there were in decay resistance, as indicated by the weight loss in the respective test blocks. The question then arises whether these variations are closely associated either with the amount of extractive in the wood or with the toxicity [8] of the extractives. These relationships can be considered with greater efficiency if the data of table 1 are first compiled by means of covariance analysis. The procedure used in making this analysis is standard (*11*); hence only the final compilations are required here, and these are given in table 2.

A comparison of the original weight-loss variances in table 2 shows that there were a number of statistically significant variations in decay resistance in the material sampled. The variations in decay resistance were associated with the radial position of the blocks in the tree (as shown in table 1, the weight loss of the wood blocks was progressively greater from the outermost to the innermost part of the heartwood), with differences in the several trees of the common locust group but not of the shipmast and Flowerfield selections, and with differences in

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[8] In this paper, the expression "toxicity of the extractives" refers to the toxicity of the total amount of extractive obtained from a 2-gm sample of wood. Hence the toxicity (as measured by fungus growth rate) reflects the influence of both the amount and the unit toxicity of the extractives in the wood.

the selections. The mean values in table 1 indicate that the Flowerfield samples showed more over-all resistance to decay than the shipmast and common samples. The extremely large variation among the common locust trees was to be expected, inasmuch as the trees in this group constituted heterogeneous and unselected types whereas the

TABLE 1.—*Relation of decay resistance* [1] *of black locust heartwood to the hot-water extractive content of the wood and to the growth rate of Fomes rimosus on malt-agar medium containing hot-water extractives in proportion to amounts present in the wood*

[Experiment 2]

Selection,[2] tree No., and position of sample (fig. 1)	Loss in weight [3]	Extractive content [4]	Linear growth [5] of fungus in 7 days
Shipmast: B6-7:	*Percent*	*Percent*	*Millimeters*
1	2.6	13.07	----
2	2.1	12.18	----
3	2.8	10.66	----
4	3.9	9.83	----
5	5.5	8.43	----
Mean	3.4	10.83	----
B10-8:			
1	1.2	12.54	----
2	2.1	10.74	----
3	1.3	9.27	----
4	3.9	6.84	----
5	4.2	4.94	----
Mean	2.5	8.87	----
Mean	3.0	9.85	----
Flowerfield: C42-7:			
1	1.3	11.53	38.0
2	.9	11.44	38.0
3	.9	11.64	36.2
4	1.0	9.92	38.2
5	1.3	9.09	39.2
Mean	1.1	10.72	37.9
B46-1:			
1	1.5	11.36	35.5
2	1.8	10.50	35.0
3	1.6	9.62	35.2
4	1.7	10.43	37.2
5	1.7	9.88	36.0
6	2.4	9.30	36.5
Mean	1.8	10.18	35.9
B46-2:			
1	0.8	12.22	33.8
2	1.3	11.06	33.8
3	1.1	10.18	34.2
4	1.1	10.34	36.0
5	1.1	9.84	33.2
6	1.5	9.82	35.5
7	1.4	9.20	34.8
Mean	1.2	10.38	34.5

Selection,[2] tree No., and position of sample (fig. 1)	Loss in weight [3]	Extractive content [4]	Linear growth [5] of fungus in 7 days
Flowerfield—Con. B46-3:	*Percent*	*Percent*	*Millimeters*
1	1.1	12.15	34.5
2	.8	10.97	35.5
3	.7	10.13	39.0
4	1.0	10.56	35.0
5	1.0	9.89	35.5
6	1.6	8.46	36.0
7	2.3	8.60	32.5
Mean	1.2	10.10	35.4
Mean	1.3	10.32	35.8
Common: B53-7:			
1	5.5	5.74	39.9
2	6.3	4.96	45.4
3	8.1	3.10	47.5
Mean	6.6	4.6	44.3
B54-7:			
1	1.1	10.40	40.1
2	1.2	9.60	38.1
3	1.1	7.70	43.2
4	1.8	6.32	43.0
5	2.8	5.87	45.4
Mean	1.6	7.98	42.0
C56-2:			
1	1.3	9.16	37.5
2	2.2	8.08	35.4
Mean	1.7	8.62	36.4
B56-2:			
1	1.2	9.44	37.0
2	1.1	7.85	39.4
3	.9	6.71	40.9
4	2.9	4.76	44.8
Mean	1.5	7.19	40.5
A54-18:			
1	2.8	12.62	39.5
2	4.1	11.36	36.0
3	4.5	10.46	35.6
4	5.3	10.14	39.1
5	3.5	9.74	34.9
6	5.0	9.14	35.4
7	5.3	8.38	34.8
Mean	4.4	10.26	36.5
Mean	3.2	8.17	39.7

[1] Decay resistance is indicated by percentage loss in oven-dry weight of the wood, caused by *Fomes rimosus*.
[2] For convenience, the common locust trees are summarized together. (See p. 416 for varieties used.)
[3] Weight-loss values are means of 2 samples.
[4] Extractive-content values are means of 2 samples.
[5] Growth rates are means of 2 cultures for the Flowerfield samples and 4 cultures for the common samples.

TABLE 2.—*Analysis of covariance of percentage weight loss caused by Fomes rimosus in relation to content of hot-water extractives and toxicity of the extractives*

[Experiment 2; calculated from data of table 1]

Source of variation	Original data		Adjusted for extractive content			Adjusted for extractive toxicity		
	Degrees of freedom	Variance	Degrees of freedom	Variance	Correlation between weight loss and extractive content	Degrees of freedom	Variance	Correlation between weight loss and linear growth
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(A) Among selections: [1]								
Shipmast, common, and Flowerfield	2	23.50**	2	11.56**				
Common and Flowerfield	1	42.17**				1	23.59**	
B) Among trees within selections: [2]								
Shipmast	1	1.76	1	6.76**				
Common	4	18.23**	4	18.10**		4	18.99**	
Flowerfield	3	.59	3	.50**		3	*.61	
(C) Among radial positions within trees: [3]	45	.72	44	.29		36	.39	
Shipmast	8	1.94**	7	.51	−0.88**			
Common	16	.88**	15	.46	−.76**	15	.59**	0.61**
Flowerfield	21	.14**	20	.09*	−.61**	20	.13**	.29
(D) Experimental error (between duplicated samples):								
Shipmast	10	.37						
Common	21	.19						
Flowerfield	25	.04						

[1] Significance of variance determined from variance ratio between A and C.
[2] Significance of variance determined from variance ratio between B and C.
[3] Significance of variance determined from variance ratio between C and D.
* Significant values (5-percent level).
** Highly significant values (1-percent level).

shipmast and Flowerfield variants were each distinct types with relatively uniform characters.

As regards the relation of extractive content and extractive toxicity to these variations in decay resistance, the extractive data in table 1 indicate that both the content and toxicity of the extractives were associated in some degree with the weight loss. These relations within individual trees are expressed by pooled linear correlation coefficients, shown in columns 6 and 9 of table 2. The extractive content is inversely correlated with weight loss, and the correlation coefficients are highly significant for trees of all three selections. Growth rate of the test fungus on the extractive medium was significantly correlated with weight loss within trees of the common locust, but within trees of the Flowerfield locust the correlation was small and not mathematically significant. Toxicity tests were not run, as mentioned previously, on the shipmast locust extractives. From the correlation coefficients obtained for the common and Flowerfield selections, it appears that weight-loss variations among radial positions within the trees were associated more closely with the extractive content than with the measured toxicity of the extractive.

The comparative utility of extractive content and extractive toxicity for indexing the observed weight loss may be further judged by the adjusted variances of columns 5 and 8 in table 2. These data in-

dicate the variation in weight loss among the selections (A), among the trees in each selection (B), and among the radial positions within the trees (C), after such variations as are related to differences in extractive content or toxicity have been accounted for. Any variation that significantly exceeds in magnitude the average variation in weight loss between duplicate tests at each radial position (D, error) can logically be attributed to variable factors other than the extractives.

The variances among radial positions within trees (C), which were highly significant for the original data, are largely accounted for by the extractive content. This is evidenced by the fact that the adjusted variances (C, column 5) are no longer significant in the case of the shipmast locust and common locust trees and barely significant in the case of the Flowerfield locust trees.

The toxicity of the extractives, on the contrary, does not give as promising a result, since the adjusted variances (C, column 8) remain highly significant for the two selections included in this test. The fact that the adjusted variances are somewhat lower than those of the original data and that there is a significant correlation coefficient for the common locust trees indicates that toxicity, as measured by the growth rates in the agar tests, is associated with weight loss, but the relation is not close enough to account for all the significant variation in weight loss among radial positions within the trees.

When the adjusted variances among the trees of the common and Flowerfield selections (B) and between the selections (A) are examined, it is seen that neither extractive content nor extractive toxicity accounts for much, if any, of these sources of variation in weight loss. This is shown by the fact that the adjusted variances among trees and among selections are but slightly or not at all smaller than the corresponding variances calculated from the original data. Moreover, in all cases, these adjusted variances are significantly greater than both the experimental error and the adjusted variance within trees.

The indications from these results, therefore, are that the proportion of hot-water extractives in the wood accounts for almost all the significant variations in weight loss within individual trees, but, on the same basis, accounts very little for differences in weight loss among different selections or among trees within selections. This result might be interpreted as an indication that the toxic composition of the extractives at different radial positions within individual trees was comparatively uniform but that among different trees and selections it was not. While the toxicity of the extractives, as measured by fungus growth rate, might theoretically be expected to index decay resistance better than extractive concentration alone, the results indicate that the opposite actually occurred; nevertheless the two extractive measures were related rather closely.

An interesting side light on the preceding results was the relation between the color of the wood and the extractive content. In general, the greater the amount of yellow in the color of the sawdust sample, the higher was the percentage of hot-water extractive. For example, samples with a light greenish-yellow color by daylight had an average extractive content of 13.30 percent, whereas the percentage in samples with a light chocolate-brown color averaged only 8.38 percent. By ultraviolet light, samples with a deep lemon-yellow color averaged 11.20 percent in extractives, whereas those fluorescing a light straw yellow averaged 8.90 percent. Intermediate colors were roughly asso-

ciated with intermediate extractive contents. These color relations, although not consistent enough to rely on as a basis for estimation of extractive content in the case of individual samples, are quite in line with the widespread belief in the greater durability of locust heartwood that is yellow in color.

CONCLUSIONS AND SUMMARY

The widely recognized superior decay resistance of black locust heartwood appears to be imparted in large measure by certain toxic chemical components. The typical high specific gravity of black locust may contribute to the general high level of decay resistance, but tests not reported here showed that this factor is little if at all related to differences in the decay resistance within this species itself. The present study shows that the hot-water extractives were not only toxic to decay fungi at concentrations considerably lower than those naturally occurring in the wood but that within individual trees they may occur in amounts and with degrees of toxicity roughly proportional to the decay resistance.

On the basis of the tests reported here with *Fomes rimosus*, it appears that the amount of hot-water extractives in the wood accounts to a large degree for the decay resistance of heartwood at different radial positions in the cross section of individual trees, but in only small measure for the differences in decay resistance among different trees or different selections of black locust. As a result of these findings it is suggested that the toxic composition of the extractive is comparatively uniform within individual trees but not among different trees.

A similar relation was generally found between decay resistance and the toxicity of the hot-water extractives as measured by the growth rate of *Fomes rimosus* on malt-agar medium containing the respective hot-water extractives in amounts proportional to the amounts present in the wood. Although theoretically a better test, the toxicity of the extracts did not index decay resistance within individual trees as accurately as did total extractive content.

The failure of the extractive toxicity to index decay resistance at least as well as the amount of extractives may be accounted for partly by the fact that the error in determining extractive content by measuring fungus rate was about three times as great as that in determining extractive content by direct weighing. Thus, even though the extractive toxicity was fully as closely related to decay resistance as extractive content, it would require a larger number of replicate toxicity tests to indicate that such was the case. It is also quite possible, particularly as regards the larger differences in decay resistance among trees and between selections, (1) that the full toxic effect of the extractives was in some manner precluded by failure of the standard method of extraction to obtain all of the toxic material in the wood, (2) that some change occurred in the original toxic material as a result of the extraction process or subsequent treatment of the extractions (this possibility was indicated by precipitates that formed in the culture medium), or (3) that certain of the extractives were sufficiently toxic to effectively retard those physiological processes of the fungus necessary to the digestion of such a complex substance as wood but not to inhibit development of the fungus greatly on a readily utilizable medium such as malt extract. That is to say, on such diverse sub-

strates as malt agar and wood, the manner in which the toxicants are effective conceivably may be basically different.

This study indicates that, with the procedures followed, the extractive content and its toxicity as measured by its effect on fungus growth in artificial culture are inadequate for a precise evaluation of the decay resistance of black locust selections involving different trees or selections. However, since a definite relation apparently exists between these factors within individual trees, it is suggested that further efforts in this direction might be profitable, possibly including tests of water extractions made without high temperatures and, in addition, extractions with nonaqueous solvents.

As a supplementary observation it was noted that the amount of yellow color in the wood, after conversion into sawdust, was roughly in the same order as the total extractive content, but the relation was not consistent enough to use as a basis of estimation in the case of individual samples. However, it may be significant that this observation is in line with the greater durability commonly ascribed to "yellow" locust.

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A RAPID METHOD FOR FINDING THE VOLUME AND DENSITY OF MUSKMELON FRUITS [1]

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INTRODUCTION

Size or volume is an important factor to be considered in variety improvement of muskmelons. The volume-weight or ratio of weight to volume, the density index, is a satisfactory indication of the amount of edible pulp—one of the important factors that determine market value. A density index obtained by cutting the fruits and classifying them by inspection is not always sufficiently accurate. Moreover, such treatment renders the fruits unsuitable for further handling or storage. To measure the volume of a large number of melons by xylometric methods is laborious and time consuming. The method outlined in 'this report combines features which might simplify the measurement of volumes and therefore density. The procedure is rapid and sufficiently accurate, with the advantage that the major part of the work can be done from the records, thereby facilitating work on a highly perishable fruit.

MATERIALS AND METHODS

Measurements from 426 muskmelon fruits that varied in size, shape, and genetic origin were used to determine the necessary data for the preparation of the muskmelon volume alinement chart. In addition, the longitudinal cross sections of 80 melons were graphically analyzed to compare the shape of muskmelons with the shape of geometrical solids of revolution generated by the circle and ellipse. Figure 1 shows the frequency distributions of the diameters, volumes, and form classes of the 426 melons. The approximate ranges of the different measurements are as follows: The smallest fruit was 8 cm. in diameter and 9 cm. in length; the largest approximately 20 cm. in diameter and 23 cm. in length. The volumes varied from 400 to over 4,500 cc., and the shape or form from very elongated fruits with a diameter 60 percent of the length to very flat melons with the diameter over 115 percent of the length. However, as shown in the frequency distributions, melons with this extreme shape were rare.

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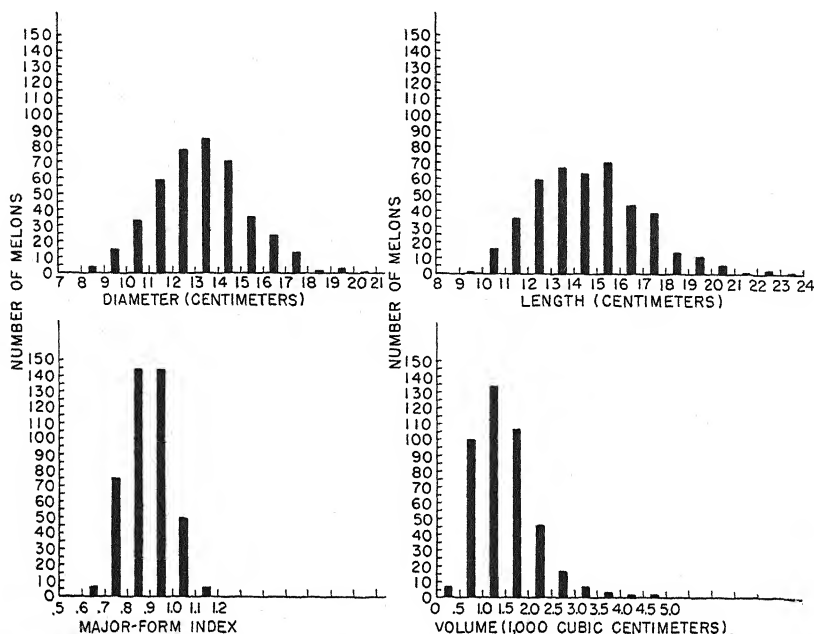
FIGURE 1.—Frequency distributions of the diameter, length, major-form index. and volume of 426 muskmelon fruits.

Most of the fruits came from strains grown on experimental breeding plots. A few were purchased locally, including Honeydew and Mildew Resistant 45 types not commonly grown in Minnesota.

It is thought that the sample adequately covers muskmelon fruit variations in size and shape, with the possible exception of very flat types which are relatively rare. Nevertheless, before applying the results to other melon populations, a test sample should be checked by a method suggested later.

The length and diameter of each melon were measured to the nearest one-tenth centimeter by the caliper shown in figure 2. To obtain an accurate average diameter, the largest and smallest diameters were measured and averaged. The volume was determined to the nearest cubic centimeter by submerging each fruit in a tank filled to an overflow tube and measuring the volume of water displaced. Weights were determined to the nearest gram on a beam balance.

In order to ascertain whether the fundamental factors that determine volume vary with the general shape of fruits, a numerical index of shape or form was necessary. For this purpose, the ratio of the diameter to the length was used as an index of general form and called the major-form index.[3] This was computed for all fruits. By means of this index, the melons were arbitrarily classified into form or shape classes of 0.675, 0.775, etc. The form factor, $\frac{V}{D^2L}$, or ratio of the actual volume to the product of the square of the diameter, D^2, times the length, L, was computed for each of the 426 fruits.

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[3] The ellipticity or $\left(1 - \frac{d}{L}\right)$ might have been used as an alternate index of form.
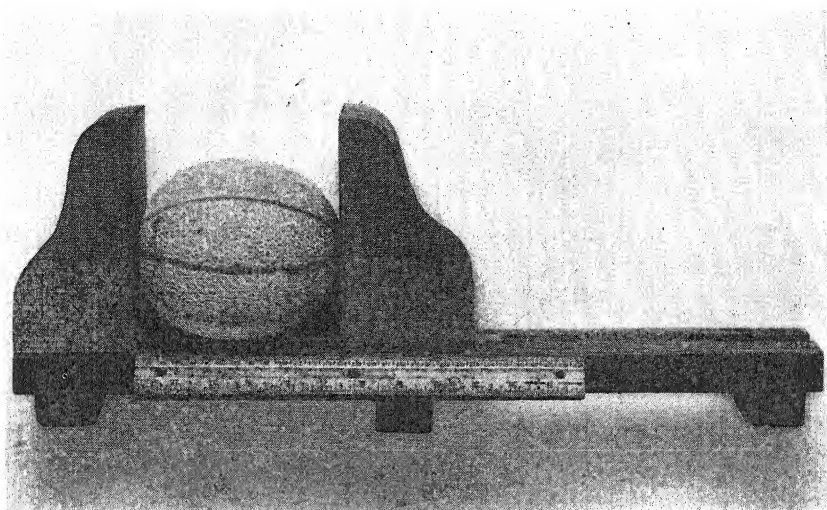
FIGURE 2.—Caliper used to measure the diameter and length of muskmelon fruits.

To study the actual shape of the muskmelons in order to compare them with the shape and volume of geometrical solids of revolution, diagrams of the major or longitudinal cross section through the blossom and stem ends were made by halving 80 melons and tracing the cross sections on paper. The major axis or length, the minor axis or diameter, and the chords at 10-percent intervals of each semilength beginning with the center of the melon as the origin of the x axis were drawn on each diagram, as shown in figure 3. Next the length of each
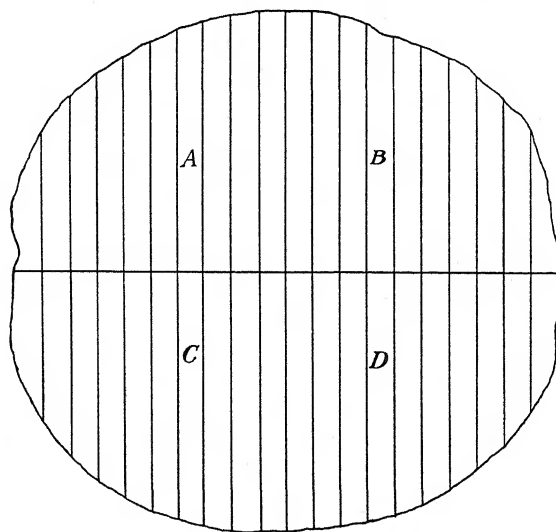
FIGURE 3.—Ordinates at 10-percent intervals of the semilength on a major cross section of a muskmelon fruit. Eighty melons were diagramed by this method to obtain the melon taper curves.

ordinate in all quadrants was measured. In order to eliminate the effect of fruit size, each absolute ordinate was expressed as a percentage of the semidiameter. These relative ordinates at each 10-percent interval of the semilength from all four quadrants were averaged for each melon. These percentages plotted over their corresponding 10-percent intervals are the relative or percentage taper curves of the melons by means of which their shape was compared with the ellipse and circle, the taper curves of the corresponding geometrical solids of revolution. The relative ordinate at the middle of each taper curve was designated the minor form index and used to group the taper curves into more precise shape or form classes.

To determine whether the cross section through the diameter of a melon could be assumed to be a circle for volume computations, the 80 test melons were analyzed as follows. A diagram of each cross section was carefully traced on paper, the long and short diameters were measured, and the difference between the averages was tested statistically. The difference of 5 percent between these average diameters was found to be very highly significant. The minor cross section of a melon is therefore elliptical rather than circular.

To determine whether this difference of 5 percent between the diameters introduced a significant error in the computation of the volume when 2 diameters were averaged, a random sample of 16 melons was analyzed by the following method: The average diameter of each melon was obtained by 2 methods—the long and short diameters were averaged, and also the long diameter and the diameter at right angles to it. The cross sections were then assumed to be circles and the areas corresponding to each of these average diameters were obtained from a table of areas of circles. Next the tracing of each cross section was planimetered twice and the areas averaged to obtain the true area for each melon. The significance of the differences between the true area and these average areas of a circle obtained by the foregoing methods was tested by analysis of variance. The difference between the area corresponding to the area of a circle with a diameter equal to the average long diameter and the one at right angles and the true area was found to be very highly significant; but the difference between the true area and the area of a circle with a diameter equal to the average of the long and short diameters was not significant. It therefore seemed apparent that the area of the cross section through the diameter of a melon could be determined accurately enough for volume computations by averaging the long and short diameters and considering the cross section a circle.

In general the method of analyzing the data was as follows: A preliminary test was made to determine the applicability to melon volumes of the formula $0.5236\ D^2L$, the basic volume formula for an ellipsoid and sphere. The volume of many of the fruits was found to differ significantly from this formula. To determine the causes of such variation, the muskmelon taper curves were compared with the ellipse and circle, the generating curves for these geometrical solids of revolution. The variation of the coefficient of D^2L, called the form factor, was investigated for the actual fruits and found to be a linear function of major-form index. The equations for the regression of form factor on major-form index were therefore derived by simple

correlation. The form factors for each major-form index were computed from these equations and these form factors used to set up volume formulas for each major-form class. To facilitate the computation of volumes, a special slide rule and an alinement chart were designed. As a means of determining how well the estimated volumes represented the actual volumes and also to determine whether the estimates from the formulas could be improved, the actual and estimated volumes were compared graphically.

MUSKMELON TAPER CURVES

The individual percentage taper curves were classified and averaged by arbitrary minor-form index or shape classes. The actual average taper percentages for each minor-form class and also the corresponding percentages for the ellipse and circle are shown in table 1 and in figure 4.
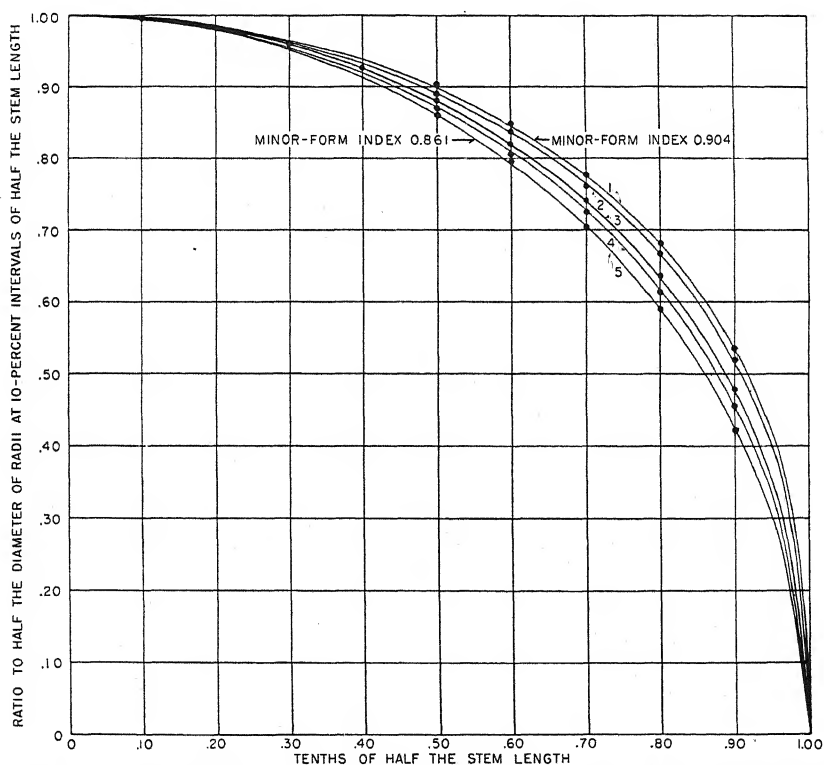
FIGURE 4.—Freehand taper curves to illustrate the general shape and character of the curves for the minor-form index classes.

The ellipsoid and sphere are solids of revolution generated by an ellipse and circle respectively. Therefore muskmelon fruits may be compared with the solids by comparing the formulas of these plane figures with the actual taper curves of the fruits.

TABLE 1.—*Comparison of percentage melon taper curves for different minor-form index classes and percentage taper curve of an elipse and circle*

Percentage of semilength [1]	Relative taper [2] (percentage of semidiameter) for minor-form index class—						Taper for ellipse and circle [3]
	0.850	0.860	0.870	0.880	0.890	0.900	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	100.0	100.0	100.0	100.0	100.0	100 0	100.0
10	98.9	99.2	99.3	99.6	99.5	99.6	99.4
20	96.7	97.8	97.9	98.3	98.4	98.6	98.0
30	94.2	95.2	95.4	96.1	96.2	97.1	95.4
40	90.4	91.5	91.6	92.6	93.2	94.2	91.6
50	85.2	86.1	87.0	88.1	89.0	90.4	86.6
60	79.2	79.5	80.6	82.0	83.8	84.9	80.0
70	70.7	70.3	72.5	74.1	76.1	77.7	71.4
80	59.0	59.0	61.4	63.6	66.7	68.2	60.0
90	43.5	43.4	45.6	47.9	52.0	53.6	43.6
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	
Melons	2	9	12	27	20	10	

[1] The percentage ordinates are given for 10-percent intervals of the semilength. beginning with the center of the melon as the origin and the semidiameter at this point as the 100-percent ordinate.
[2] Actual uncurved averages.
[3] The minor-form index of an ellipse and circle is constant, 0.866.

COMPARISON OF THE SHAPE OF MELONS WITH GEOMETRICAL SOLIDS OF REVOLUTION—THE ELLIPSOID AND SPHERE

The basic formula for both the ellipse and the circle is

$$\frac{y^2}{B^2}+\frac{x^2}{A^2}=1, \text{ or } \left(\frac{y}{B}\right)^2+\left(\frac{x}{A}\right)^2=1 \tag{1}$$

where x and y are the rectangular coordinates of any point on the perimeter of the plane figure, B is the semiminor axis or one-half the diameter and A is the semimajor axis or one-half the length. When A equals B, the two axes are equal and the formula reduces to the equation for a circle. The second form of the equation shows that the sum of the squares of the relative or percentage ordinates and relative abscissas is equal to unity. This fact might have been used to compare the ellipse and circle with the melon taper curves.

The first comparison of interest is the comparison of the minor-form indexes of the melons and the circle and ellipse. If the basic formula (1) is solved for the minor-form index, it will be found to be 0.866 for both the ellipse and circle, as shown in table 1. In other words, the ordinate at one-half the semilength of an ellipse and at one-half the radius of a circle is a constant percentage, 86.6 percent, of the semi-diameter. On the other hand, as shown in table 1, the minor-form index of the melons is not constant, but may vary from 0.85 or slightly less than that of an ellipse and circle, to over 0.90, a minor-form index greater than that for the mathematical figures. In other words, the relative ordinates, and therefore the absolute ordinates of some melons, may be more or less than those of an ellipse and circle. In terms of volume, this means that a melon may have more or less volume than a corresponding solid of revolution generated by an ellipse or circle; and, for this reason, the volume formula, 0.5236 D^2L, for these solids will not give accurately the volumes of melons unless the formula is modified.

Furthermore, the minor-form index of the melons appears to vary with the major-form index. As shown in figure 5, it increases as a positive linear function of the major-form index. This trend was
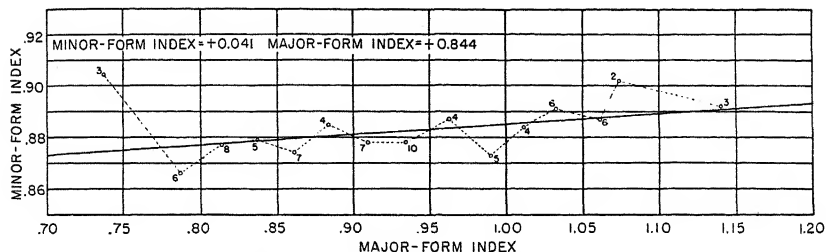
FIGURE 5.—Diagram showing the positive and significant linear correlation between the minor- and major-form index and the equation determined by correlation.

found to be almost highly significant by a statistical test of the correlation coefficient, $+0.274$. This indicates that such a relationship probably does exist in this sample of fruits. The linear equation for this line derived by simple correlation is

Minor-form index$= +0.041$ major-form index $+0.844$　　(2)

Thus as the ratio of the diameter to the length of melon increases, the form, and therefore the volume of melons, increases with major-form index. This necessitates changing the constant, 0.5236, in the volume formula of an ellipsoid for changes in the major-form index of a melon.

To determine whether the melon taper curves were mathematically similar throughout their length to the taper curves of an ellipse and circle, they were compared graphically by the use of logarithmic cross-section paper. If the formula for these geometrical figures is solved for a relative ordinate, it reduces to the form of the well-known parabolic equation, $Y = PX^n$, which plots as a straight line on double logarithmic cross-section paper. The ellipse formula reduced to this type is:

$$\frac{y}{B} = \left[1 - \left(\frac{x}{A} \right)^2 \right]^{\frac{1}{2}} \tag{3}$$

where $\left[1 - \left(\frac{x}{A} \right)^2 \right]$ corresponds to x in the parabolic formula and $\frac{1}{2}$ to n, and p is equal to 1. If logarithms are taken of both sides of this equation, it reduces to the following linear form:

$$\log \frac{y}{B} = \frac{1}{2} \left\{ \log \left[1 - \left(\frac{x}{A} \right)^2 \right] \right\} \tag{4}$$

The relative taper curves for the melons may therefore be compared with the ellipse and circle by plotting the melon taper percentages $\frac{y}{B}$ in table 1, which correspond to $\frac{y}{B}$ in the formula, over $\left[1 - \left(\frac{x}{A} \right)^2 \right]$ on double logarithmic cross-section paper. If the melon curves are based
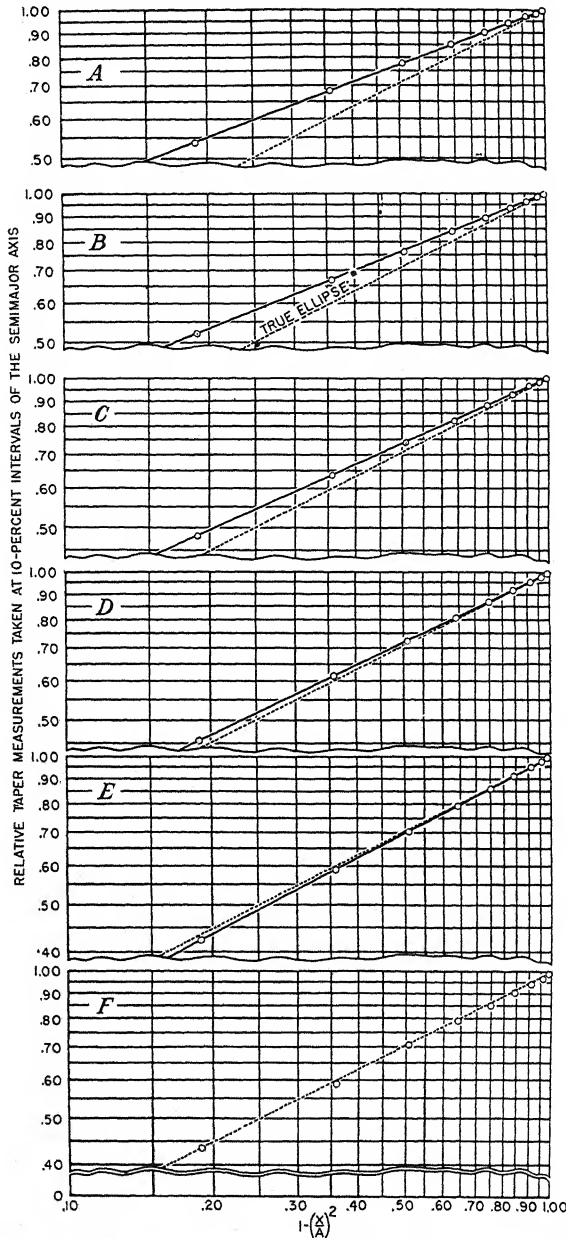
FIGURE 6.—The raw average percentage taper curves of the 80 test melons plotted over $\left[\left(1-\dfrac{x}{A}\right)^2\right]$ on double logarithmic cross-section paper. The absence of any systematic curvature in these lines shows that they are parabolic curves of the general type, $y=px^n$; and, therefore, the melon curves are members of the same mathematical family of curves to which the ellipse and circle belong. *A*, Minor-form index 0.904, 10 melons; *B*, minor-form index 0.890, 20 melons; *C*, minor-form index 0.881, 27 melons; *D*, minor-form index 0.870, 12 melons; *E*, minor-form index 0.861, 9 melons; *F*, minor-form index 0.852, 2 melons.

on the same type of mathematical equation, they will plot as straight lines.[4]

The actual average taper percentages given in table 1 for each minor-form index class are plotted in this manner in figure 6.

As shown in figure 6, the melon taper percentages for all the minor-form index classes are straight lines. This was also true of a number of the taper curves for individual melons. This graphic test shows that the relative taper curves of melons in this random sample may be represented by the same basic parabolic equation as for the ellipse and circle, as follows:

$$Py = [1 - P_x^2]^n \tag{5}$$

where n is the general exponent and also the slope of the line on double logarithmic cross-section paper, and Py and Px are the percentage ordinates and abscissas respectively. Since the slopes of some of these straight lines are less and others greater than the ellipse as shown in figure 6, the exponent n is variable and more or less than one-half.

DERIVATION OF A VOLUME FORMULA FOR MUSKMELON FRUITS

Derivation of a volume formula from this basic parabolic equation by calculus showed that the variation in the general exponent, n, in this basic formula merely varied the coefficient of D^2L in the ellipsoid volume formula, $0.5236D^2L$, but not the exponents of D and L.[5] The ellipsoid formula may therefore be generalized for melons by substituting a variable, K, called the form factor, for the constant 0.5236.
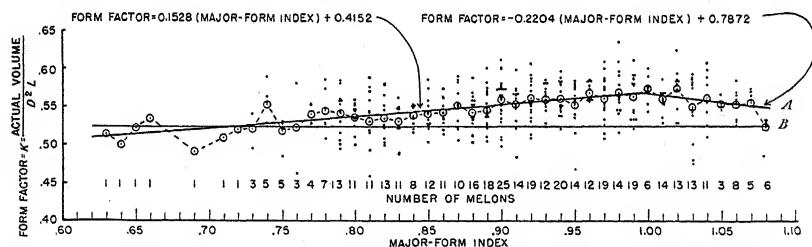
FIGURE 7.—Regression of the form factor, K, of the muskmelon volume formula, KD^2L, on the major-form index.

The formula may be written as follows: Volume-KD^2L. The actual value of K was computed for each fruit by dividing the volume by D^2L. Because the minor-form index was found to be a function of the major-form index (fig. 5), K likewise could be expected to be a function of the major-form index. To determine the form of this relationship, K was plotted over the major-form index; and, as shown in figure 7, the trend was found to be a positive linear function up to a major-form index of 1, but above this it was negative. The two correlation coeffi-

[4] BEHRE, C. E. FORM-CLASS TAPER CURVES AND VOLUME TABLES AND THEIR APPLICATION. Jour. Agr. Res. 35 : 673–744, illus. 1927.
[5] The variation of the exponents of D and L in the general volume formula, KD^mL^n, was investigated by deriving for each major-form class the unknowns K, M, and N by multiple linear correlation of the logarithms of diameter, length, and volume, but this method was dropped when it was found that the estimates were not enough better to justify the use of the more complex estimating method.

cients, $+0.422$ and -0.296, were highly significant statistically. Therefore within the limits of this sample, the relationship between the form factor K and the major-form index may be represented by straight lines. The equations for these lines derived by simple correlation are

$$\text{Form factor } (K_1) = +0.1528 \text{ major-form index } +0.4152 \quad (6)$$
$$\text{Form factor } (K_2) = -0.2204 \text{ major-form index } +0.7872 \quad (7)$$

From these equations, the volume formula for each major-form class was obtained. For example, the form factor for the 0.6 form class is (0.1528) (0.6) $+0.4152$ or 0.5069, and the volume formula for this class is $0.5069D^2L$.

THE STANDARD ERRORS OF ESTIMATE OF THE FORM FACTOR K AND THE VOLUME

The actual volumes vary from the estimated volumes for several reasons. The average taper curve in one cross section in any one melon may differ from the curve in another cross section. Even within a given cross section, the taper curves may vary from quadrant to quadrant; and the melon, therefore, may not be a perfect solid of revolution. Melons in a given major-form index class may have taper curves that vary from the average for the class. The cross section through the diameter of any individual fruit may be significantly different from the area of the circle or ellipse that corresponds to the average diameter. Different varieties differ in form and therefore in volume. These variations result in errors of estimate for both the form factor K and the volume. The errors of estimate for the equations for K are respectively ± 4.5 and ± 5.1 percent or approximately ± 5 percent. Inasmuch as the volumes are directly proportional to K, the average standard error of the computed volume for any individual melon selected at random from 426 should also be ± 5 percent. Since very few large flat fruits were available, a reliable estimate of the standard error of this type could not be determined.

Although neither a graphic nor a statistical analysis was used directly to determine whether the form factor varied with the size of the melons within a major-form index class, conclusions may be drawn indirectly from the trends of the lines fitted to the actual volumes plotted over estimated volumes independently classified by diameter, by length, and by volume. If the trends are represented by 45° straight lines through the origin, the form factor does not vary with these variables. As illustrated by figure 8 for two major-form classes, the trends may be represented by this 1 to 1 slope. With the exception of large, very flat melons for which sufficient data were not available, this was also true for the other form classes. Therefore different form factors need not be used for melons with different diameters, lengths, or volumes in the different major-form index classes.

MELON VOLUME SLIDE RULE

In an effort to facilitate the computation of the estimated volume, an adaptation of an inexpensive wooden slide rule was used. The D scale was used for diameters, the B scale for lengths, and the A scale
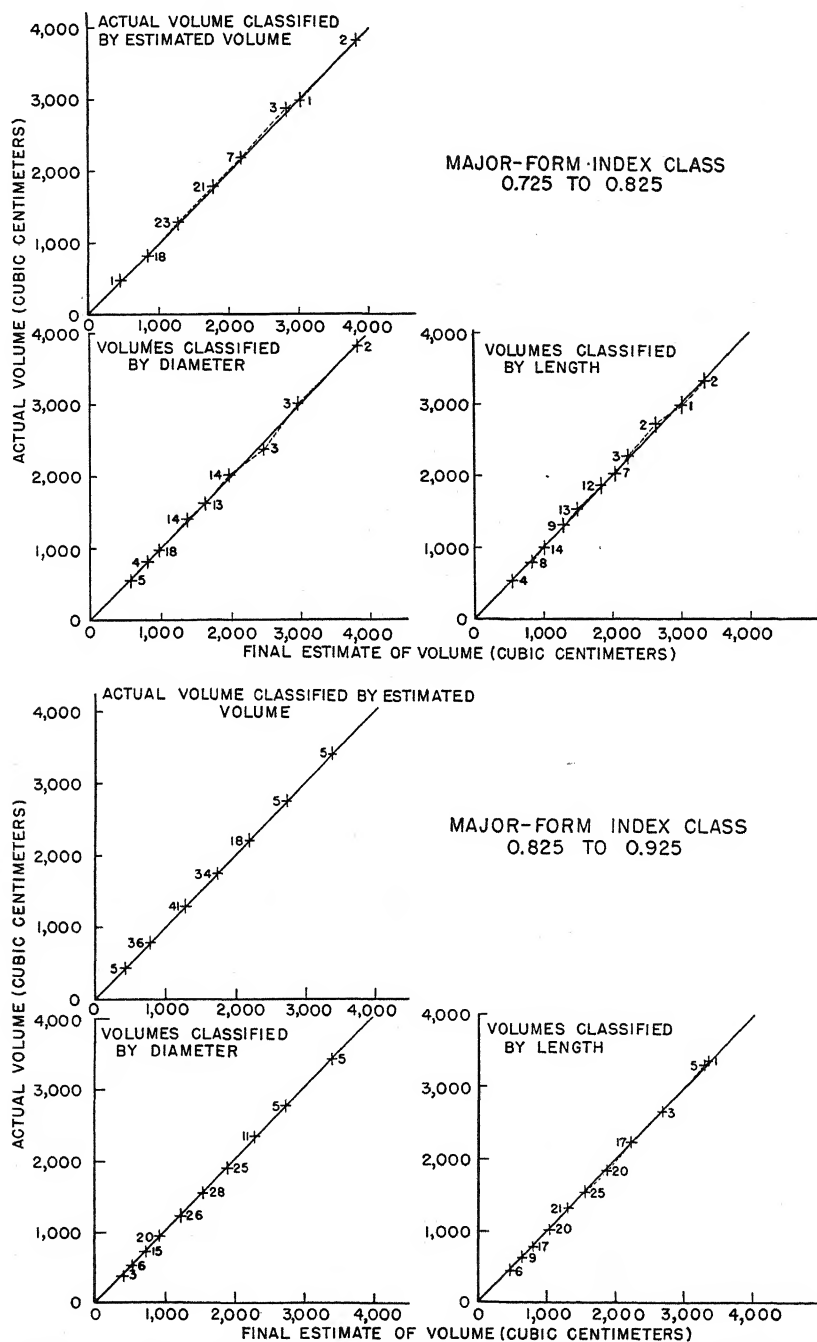
FIGURE 8.—Comparison of actual and estimated volumes of muskmelon fruits in two major-form classes.

for volumes. The only modification necessary was the addition of a major-form index scale among the C scale divisions. These divisions may be inked with colored ink to distinguish them from the regular C scale divisions. Because the divisions for major-form indexes greater and less than 1 overlap, they were also marked with different colored inks.

The form-index scale was inserted by assuming any convenient melon length, such as 20 cm. This length was multiplied by each major-form index, 0.6, 0.7, etc., to obtain the corresponding diameters. Then the form factor, K, for each major-form index was computed from formulas 6 and 7. From K and the diameters and lengths, a volume for each form index was calculated. Next a scale division was located on the C scale for each form index, as follows: The runner was set on the volume on the A scale and the length on the B scale was brought to the runner; the runner was then set on the diameter on the D scale, and under the runner on the C scale the division for the given form index was inserted. This was repeated for each major-form index.

The major-form index, the volume, and the density of muskmelon fruits may be computed by determining the length and diameter with the average diameter being taken as midway between the longest and shortest diameters. The major-form index is computed on the C and D scales by dividing the diameter by the length. The runner is now set to the diameter on the D scale and the form index is brought to the runner. The runner is shifted to the length on the B scale and the estimated volume is indicated under the runner on the A scale. The right index on the rule will have to be reset to the position of the left index when the rule is used to compute the volumes of fruits with diameters and lengths less than 10 cm. To compute the density, the weight of the fruit is divided by the estimated volume on the C and D scales.

VOLUME ALINEMENT CHART

A simple alinement chart [6] as illustrated in figure 9 has certain advantages over the slide rule. Errors are less likely to occur since the chart can be easily read by inexperienced individuals. It is a rapid method for computing the volumes from the formula KD^2L, especially if K is variable. To find the volume of a muskmelon, the diameter and length are measured and the major-form index computed. For example, the major-form index of a melon with a diameter of 9 cm. and a length of 11 cm. is 0.82. A straightedge is placed to intersect the diameter, 9, and the length, 11, as shown by the line A in figure 9. While the point of intersection on the dummy axis is held with a pointed instrument, the free end of the straightedge is passed through the major-form index, 0.82, as represented by line B. The volume, 480 is read where the straightedge intersects the volume axis. From this volume and the weight of the fruit previously determined, the density is computed, and can be used to indicate the extent of the seed cavity in the fruit.

[6] Bruce D. and L. H. Reineke: Correlation alinement charts in forest research. U. S. Dept. Agr. Bul. 210, 1931.
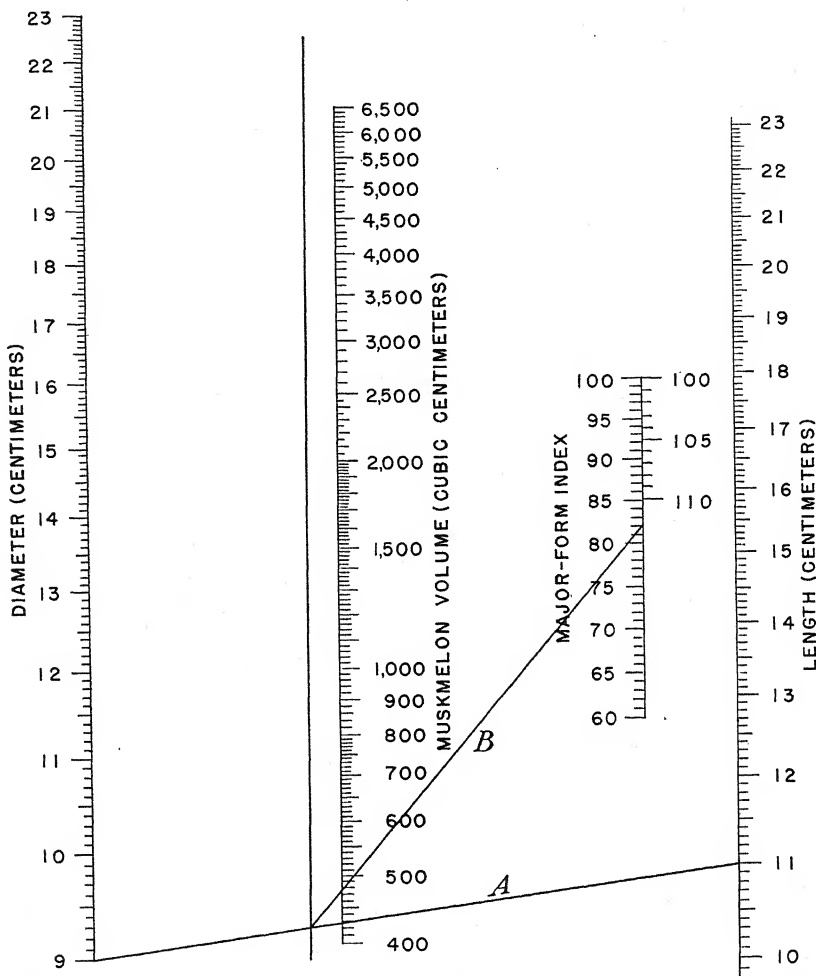
FIGURE 9.—Alinement chart to obtain the volume of muskmelon fruits from length, diameter, and major-form index.

COMPARISON OF THE ACTUAL AND ESTIMATED VOLUMES

To determine how accurately the formula estimated the volume of melons of different diameters, lengths, and volumes in a given major-form index class, and also to determine whether or not these estimates could be improved, the melons were classified into major-form index classes, 0.625 to 0.724, etc. Within each class, the fruits were grouped into diameter classes irrespective of length and volume, and the actual volumes plotted over the corresponding estimated volume for each diameter class. This process was repeated for both length and volume. With the exception of a few of the largest melons in the highest form class, the trend of the lines was found to be a 45° straight line through

the origin, as shown in figure 9 for two major-form classes. From this it is evident that the average actual volumes are equal to the average estimated volumes for melons of different shapes and sizes. Therefore it seems that no further improvement in the estimates of volume could be obtained for this sample by this estimating procedure. It should, however, be noted that the lack of data for large, flat fruits eliminates any assurance that the chart and slide rule will estimate volumes of this shape and size with sufficient accuracy.

The chart may not estimate volumes of all populations with consistent accuracy. It may, however, be tested and, if necessary, adjusted by the following method: Plot the actual over the estimated volumes for a test sample of not less than 25 fruits, and if the trend may be represented by a 45° straight line through the origin, the chart need not be changed. If it is a straight line other than 45°, the significance of the deviation can be tested by the pairing method, and if the differences are significant, the chart should be changed. In the event that the trend is a curve, the deviation from a 45° straight line may likewise be tested statistically. When the curvature or slope difference is significant, the volume graduations should be modified as follows: If an average actual volume of 456 corresponds to an average estimated volume of 450, the volume graduation, 450, on the chart is labeled 456. By reading additional volumes from the trend lines of actual over estimated volumes, a skeleton series of revised volume divisions are located on the volume axis. To interpolate additional divisions, the vertical distances to these new divisions may be plotted over the new volume graduations and a smooth curve drawn through the points. The revised volume scale is then completed by transferring divisions from this graduating curve. After such revision, the chart should give the desired accuracy for the population from which the sample was taken.

The slide rule may also be modified by relocating the major-form index scale divisions.

SUMMARY

Since the density of muskmelon fruits indicates the extent of the seed cavity, an attempt was made to develop a mathematical formula to determine the volumes of fruits from a mixture of muskmelon strains. The shape and volume of 426 melons were studied analytically and compared with the shape and volume of an ellipsoid and sphere in order to determine whether the volume formulas for these geometrical solids could be adapted to muskmelon fruits. Volume formulas for melons of different shapes were derived. In order to best utilize the formulas, a slide rule and an alinement chart were designed. A graphic comparison between actual and estimated volumes suggests that the volumes of fruits in the sample were generally estimated with sufficient accuracy.

SOME ROOT ROTS AND A FOOT ROT OF LUPINES IN THE SOUTHEASTERN PART OF THE UNITED STATES [1]

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INTRODUCTION

For a number of years agronomists of certain southern agricultural experiment stations have grown three species of lupines (*Lupinus* spp.) experimentally, to determine whether they are suitable for use as winter cover crops. One of these species (*L. angustifolius* L.) is now being grown commercially, more extensively each year. The experimental and limited commercial plantings have brought out the fact that, vigorous as these plants appear to be, they are susceptible to certain diseases. The cause of the most serious of these diseases and the extent of damage to be expected from them have been under investigation for the past 4 years. Two papers by the writer (*17, 18*),[3] dealing, respectively, with an anthracnose and a botrytis disease, have been published recently. A preliminary report on a fusarium hypocotyl rot was made some time ago (*16*).

These investigations have shown that some of the diseases of lupines in this country are the same as or are very similar to those affecting lupines in other countries. Gould (*5*) has reviewed the literature dealing with certain diseases of lupines in the United States and elsewhere. Other papers, discussing these diseases in foreign countries, have been published by Fischer (*4*), Richter (*10, 11*), Dippenaar (*3*), Noll (*9*), Carrera and Noll (*2*), and many others. Since a comprehensive review of the literature is available in these papers, no attempt will be made to review it here.

ROOT ROTS

GEOGRAPHIC DISTRIBUTION AND ECONOMIC IMPORTANCE

In the spring of 1939 a decay of the roots and hypocotyls of plants of *Lupinus luteus* L. growing in nursery rows at Quincy, Fla., was called to the writer's attention by J. D. Warner.[4] Since that time root rots have been discovered at Gainesville, Fla., Tifton and Americus, Ga., and Auburn, Ala., and have been found to attack *L. albus* L. and *L. angustifolius* as well as *L. luteus*. Similar diseases have been reported from Europe by Richter (*10*), Schultz (*13*), Anagnosto-

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poulos (*1*), and others, and from South America by Carrera and Noll (*2*).

The wide distribution and severity of root rots, as reported by different investigators, justify the conclusion that they are of considerable economic importance. Where root rots have been observed by the writer, their behavior has been somewhat erratic. For example, they were first observed in severe form at Quincy, Fla., on *Lupinus luteus*, but apparently they have never caused serious damage to *L. angustifolius*, the species grown most extensively there. On the other hand, root rots have killed many or all of the plants of the latter species in some nursery rows at Gainesville, Fla., and at the same time they have caused only slight damage to *L. luteus* at Gainesville. *L. luteus* growing at Americus, Ga., has appeared to be very susceptible, but, so far as observed, commercial fields of *L. angustifolius* grown in south Georgia have as yet suffered only minor losses from root rots.

METHODS

Isolations and cultural studies were made largely on plain oat agar or 2-percent dextrose-potato agar. Cultures were grown in diffused light at room temperature, usually before a north window. Some of the inoculation experiments were conducted in the greenhouse during the winter months and others out of doors during the summer. The diseases were produced about equally well under both conditions. The soil used for growing the plants was a sandy loam contained in 6-inch pots and sterilized for 3 hours at 25 pounds' pressure. The seeds were immersed for 15 minutes in mercuric chloride (1:1,000) and washed in running water before being planted. Since some of the seed was too old and some too hard, germination was often poor, and consequently the number of plants used in many of the experiments was small, as shown in table 1. The seed or soil was usually inoculated with nodule-forming bacteria from commercial cultures. Inoculations were made by removing the soil from about the plants, usually when they were 1 to 2 inches tall, placing a few grains of oats from flask cultures of the fungus against the hypocotyl, and then replacing the soil. In many cases the fungus used in the inoculations was recovered and in several instances the pathogenicity of the reisolate was proved. Control plants, which were always included in the experiments, were treated in the same manner as the inoculated plants except that sterile oats were used instead of inoculated oats. In only a few instances did infection take place in the control plants, and then the trouble was a damping-off of the seedlings, caused by *Rhizoctonia*.

SYMPTOMS

Lupine plants affected with root rots vary somewhat in appearance. Dwarfing and often yellowing, followed in late stages by wilting and slow death, are characteristic of the top symptoms. The hypocotyl and roots show a dry rot that ranges in color from water-soaked to straw-colored, mahogany red, chestnut, chocolate, and almost black,[5] depending on the stage of development, the moisture content of the tissue, the host species attacked, and the causal agent. At first only a small superficial reddish or brown lesion appears on the affected part, but this may spread until the entire underground part of the plant is

[5] Insofar as practicable, all color names are those given by Ridgway (*12*).

involved and the plant is greatly dwarfed or wilts and dies. These diseases are not typical fusarium wilts, although wilting does occur as a result of the severance of the hypocotyl or the destruction of the root system.

Inoculation experiments have shown that several fungi cause rotting of lupine roots and the symptoms vary somewhat, as stated above. Additional descriptive notes will appear under the discussion of the inoculation experiments.

ETIOLOGY

Root rots of lupines can be caused by several different fungi, largely *Rhizoctonia solani* Kühn and species of *Fusarium*. Only a limited number of plants were available for making isolations, and it is thought that more fungi may be involved in this disease complex than are discussed here. This is suggested by the number of root-rotting fungi described by other investigators and also by the fact that fungi not isolated from lupines produced infection, as will be brought out later.

RHIZOCTONIA SOLANI

Rhizoctonia has been isolated only occasionally from naturally infected plants from the field and then it was associated with one or more species of *Fusarium*. Inoculation experiments have shown that this fungus is capable of causing a rapid decay of the hypocotyl of young lupine plants. The following experiments are typical.

On July 11, 1941, 12 seedlings of *Lupinus luteus* about an inch tall, growing out of doors in sterilized soil in 6-inch pots, were inoculated with *Rhizoctonia* originally isolated from *L. albus*. On July 14, 10 of the 12 plants inoculated were nearly dead and the underground parts were all badly decayed (fig. 1). The affected tissue was soft and water-soaked or light brown in color. Microscopic examination showed the decayed tissue to be permeated with large vigorous strands of *Rhizoctonia* mycelium. None of the control plants were affected. *Rhizoctonia* caused a typical damping-off of these plants in 3 days. Six plants of *L. angustifolius* were inoculated in the same manner at the same time. All 6 became infected, 2 being dead and the others more or less decayed on July 25. Seedlings of this species did not succumb so rapidly as did those of *L. luteus*. In like manner 10 plants of *L. albus* were inoculated on July 14, and 9 were nearly dead on July 25. In no case did the controls become infected. In other experiments, where older plants were inoculated, there was little or no infection. The results of the experiments lead to the conclusion that *Rhizoctonia* is potentially capable of causing a serious damping-off of seedlings but usually is not a very active parasite of older plants. Of course, under extremely favorable environmental conditions, the fungus might cause considerable damage to older plants.

FUSARIUM SPP.

Several different species of *Fusarium* cause root rots of lupines in other countries (*2*), and the work reported here shows that this is true also in the United States. In fact, the lupines are so commonly attacked by fusaria that it is difficult to determine which species most often acts as the primary parasite and which as the secondary invader. Over a period of several years, however, *Fusarium oxy-*

FIGURE 1.—Hypocotyl rot of *Lupinus luteus* seedlings, resulting from inoculation with a pure culture of *Rhizoctonia solani*. The decayed tissue was soft and had a water-soaked or light-brown color, in contrast to the red color of the dry rot caused by most species of *Fusarium*. Photographed 3 days after inoculation. × 1.

TABLE 1.—*Infection resulting from the inoculation of 3 species of lupines with various isolates of Fusarium from decaying lupine roots from different sources*

Isolation No.	Lupinus albus plants			Lupinus luteus plants			Lupinus angustifolius plants			Species of Fusarium	Source of isolate
	Inoculated	Infected		Inoculated	Infected		Inoculated	Infected			
	Number	Number	Percent	Number	Number	Percent	Number	Number	Percent		
1123E	8	0	0	9	6	66.7	-----	-----	-----	F. oxysporum f. radicis-lupini.	Gainesville, Fla.
1123E	7	0	0	4	2	50.0	-----	-----	-----	----do----------	Do.
1135B	9	5	55.6	7	2	28.6	-----	-----	-----	----do----------	Do.
1135B	9	0	0	2	2	100.0	-----	-----	-----	----do----------	Do.
1228C	2	0	0	1	1	100.0	-----	-----	-----	----do----------	Do.
1141D	10	8	80.0	7	6	85.7	-----	-----	-----	----do----------	Quincy, Fla.
1141D	8	1	12.5	7	3	42.9	19	9	47.4	----do----------	Do.
1141E	9	3	33.3	9	2	22.2	-----	-----	-----	----do----------	Do.
1169E	10	3	30.0	8	7	87.5	-----	-----	-----	----do----------	Reisolate of 1141D.
1202C	8	2	25.0	7	0	0	-----	-----	-----	----do----------	Do.
1231C	7	7	100.0	5	5	100.0	19	16	84.2	----do----------	Americus, Ga.
1180	10	9	90.0	8	6	75.0	-----	-----	-----	----do----------	Cotyledons.
1203	9	8	88.9	6	6	100.0	-----	-----	-----	----do----------	Reisolate of 1180.
1237B	9	5	55.6	0			-----	-----	-----	----do----------	Tifton, Ga.
1238A	6	6	100.0	3	2	66.7				F. moniliforme	Auburn, Ala.
1238B	9	9	100.0	3	3	100.0	19	17	89.5	----do----------	Do.
1229A	10	10	100.0	4	4	100.0	18	17	94.4	F. solani f. lupini	Gainesville, Fla.
1240	-----	-----	-----	3	3	100.0	17	17	100.0	F. solani f. pisi	Experiment, Ga.
1240	-----	-----	-----	22	13	59.1				----do----------	Do.

sporum Schlecht. has been isolated most frequently from lupines collected by the writer or sent to him by others. Some isolates proved to be virulent pathogens, others weak ones, and still others were nonpathogenic. Isolations have been made from diseased hypocotyls of lupine plants of different ages and from various sources. Several of the isolates have been pure-lined and used for cultural and inoculation studies. The results of some of the inoculation experiments are summarized in table 1. Three different species of *Fusarium* isolated from lupine proved to be pathogenic, namely, *F. oxysporum*, *F. moniliforme* Sheldon, and *F. solani* (Mart.) Appel and Wr. The results of the studies with these will be discussed separately.

FUSARIUM OXYSPORUM

The data in table 1 show that the isolates of *Fusarium oxysporum* from diseased plants from Gainsville, Fla., possessed different degrees of pathogenicity, seemingly being more pathogenic on *Lupinus luteus* than on *L. albus*. Isolates of the same fungus obtained from Tifton and Americus, Ga., also proved to be pathogenic (fig. 2). Cultures obtained from *L. luteus* from Quincy, Fla., proved to be pathogenic on all three species of lupines in the several tests made. In some instances a fairly high percentage of infection was obtained in one species of lupines and little or none in the others. Cultures 1169E and 1202C, which were reisolates of 1141D, and culture 1203, a reisolate of 1180, were all more or less pathogenic, as were the original isolates. Culture 1180 was isolated from cotyledons of *L. albus* after the seedlings were up but before the seed coat had pulled away from the cotyledons. The fact that this isolate was pathogenic indicates that the fungus was seed-borne. If this fungus is seed-borne, it might account for its wide distribution in a territory where lupine culture has just started.

Of 115 *Lupinus albus* plants inoculated with *Fusarium oxysporum*, 44.3 percent were infected (table 1). The same fungus infected 60 percent of the *L. luteus* plants and 65.8 percent of the *L. angustifolius* plants inoculated. These values do not suggest any marked difference in the susceptibility of the different species. The only explanation of the great variation in the amount of infection obtained in the different experiments seems to be that the fungus is very sensitive to environmental conditions. When conditions are very favorable it is highly pathogenic, but when they are less favorable it is only weakly pathogenic or not at all. This possible explanation is supported by field observations. In some years and under some local conditions this root rot is very destructive, whereas in other years there is little disease or the disease, though present, is of little economic importance.

The nature of the lesions caused by the fungus on *Lupinus luteus* is shown in figure 2. The consistent origin of the lesions on the hypocotyl is due to the fact that the inoculum was placed against the plant just below the surface of the soil and not because that part of the plant is necessarily more susceptible. Figure 2, *E*, shows that the entire root system as well as the hypocotyl may be destroyed. The figure also illustrates the fact that dwarfing of the plants and shriveling and dying of the leaves do not become conspicuous until the hypocotyl or root system is badly decayed. Figure 3 shows the same type of symptoms produced on *L. albus* by this fungus

(1231C, isolated from a plant collected at Americus, Ga.). There was no dwarfing in the two plants shown in figure 3, *A* and *B*, but another plant was considerably stunted (fig. 3, *C*). The plants were photographed 15 days after inoculation. Sometimes the fungus continues its activity until the entire root system is destroyed and
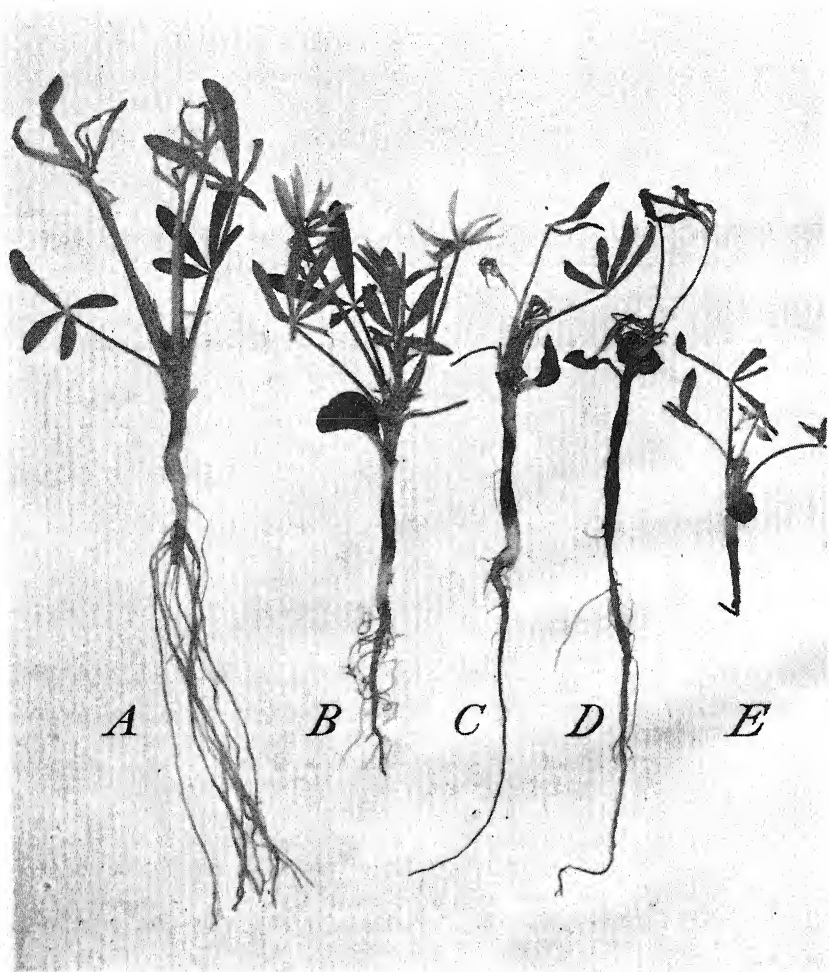
FIGURE 2.—Hypocotyl rot of *Lupinus luteus* plants, resulting from inoculation with *Fusarium oxysporum* f. *radicis-lupini: A,* Uninoculated control; *B* to *E,* plants showing various degrees of decay, from slight canker (*B*) to complete destruction of root and lower stem (*E*). × ⅗.

the plant is killed; at other times it produces only a shallow lesion, after which it ceases its activity and the decayed tissue cracks, owing to the growth of the tissue beneath, and much of it sloughs off. At first only the cortex is attacked, but if conditions are favorable for the continued action of the fungus the vascular region is invaded and may be entirely decayed (fig. 2, *E*).

The roots and hypocotyls of naturally infected plants show all

stages of decay, from very slight superficial lesions to complete severance of the underground parts at some point or the entire destruction of the fibrous root system.   The coloring in the decaying tissue resulting from natural infection, like that due to artificial
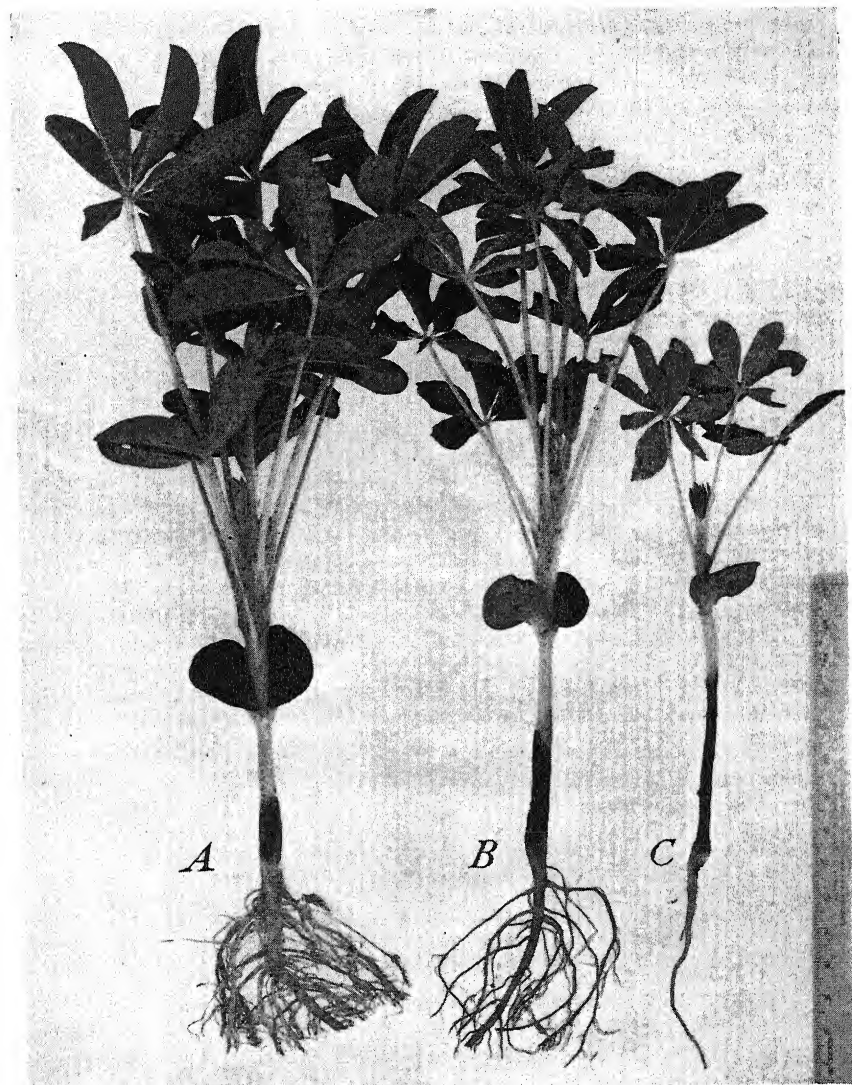
FIGURE 3.—*Lupinus albus* plants showing cankers characteristic of root rot caused on this host by *Fusarium oxysporum* f. *radicis-lupini*: *A* and *B*, No dwarfing or wilting present; *C*, considerable dwarfing and decay of hypocotyl but no wilting. $\times \frac{1}{2}$.

inoculation, varies considerably, but, as already stated, it is some shade of red or brown.   The rate at which the fungus spreads through the tissues varies greatly in both naturally and artificially infected plants.   Under field conditions, infected plants may be killed in

the seedling stage or may die at any time during the growing season. Some plants may live and mature seed, even with more or less of their root system destroyed. A stand of plants may appear entirely normal, so that the presence of the disease is not suspected until some plants are pulled and the roots examined.

There is some variation in the isolates of this species of *Fusarium*. The isolate most commonly obtained from naturally infected plants is *F. oxysporum* Schlecht. emend. Snyder and Hansen (*14*).[6] Snyder and Hansen have pointed out that there is such a wide variation in the physiological reactions, types of sporulation, and spore size in the single-spore isolates from cultures of fusaria that it hardly seems worth while to include here any detailed data of that sort. However, since some workers may wish to have a clearer idea of the relation of the fungi studied by the writer to the species listed by Wollenweber and Reinking (*19*), the following statement of Snyder[7] is included: "Since it [fungus 1141D] fails to produce sporodochia it might be placed according to the Wollenweber system in *F. orthoceras* or thereabouts."

Since this fungus belongs to the species *Fusarium oxysporum*, according to Snyder and Hansen's classification, and is parasitic on lupines, it should be designated by the form name of the host, namely, *lupini*. However, this form name has already been applied (*14, p. 66*) to a member of this species that produces a typical vascular wilt of lupines in Europe. The fungus considered herein produces a typical root rot and not a vascular wilt. It is true that some fusarium-wilt-producing fungi may cause a certain amount of root rot and that the wilt-producing fungi do not always pass up the stem for any great distance. Perhaps the difference between the wilt-producing fusaria and those causing root rot is one of degree rather than kind. If this is true, then the fungus under discussion may be called *F. oxysporum* f. *lupini*. Until further study has shown that these are intergrading forms, however, the writer feels that a different form name is justified. The name *Fusarium oxysporum* f. *radicis-lupini* n. f. is proposed.

Fusarium oxysporum f. radicis-lupini n. f.

Microconidia abundant, macroconidia usually few, typically three-septate, straight or slightly curved; nonseptate, $5\mu–13\mu \times 3\mu–4\mu$ (average $9\mu \times 3.5\mu$); one-septate, $11\mu–22\mu \times 3\mu–4\mu$ (average $14.5\mu \times 3.6\mu$); two-septate, $17\mu–20\mu \times 3.5\mu–4\mu$ (average $18\mu \times 3.7\mu$); three-septate, $17\mu–33\mu \times 3.5\mu–4.0\mu$ (average $24.5\mu \times 3.8\mu$). Aerial mycelium usually well developed; at first white; later, on old oatmeal-agar slants, dull dusky purple to dark napthalene violet. Pionnotes and sporodochia absent, no sclerotia seen. Chlamydospores abundant, terminal or intercalary, globose to pear-shaped, smooth or roughened, mostly one-celled, sometimes two-celled. One-celled, $6\mu–10\mu \times 3.5\mu–7.5\mu$ (average $7.7\mu \times 6.3\mu$). Causes a root rot of *Lupinus* spp. in the southeastern part of the United States.

FUSARIUM MONILIFORME

Fusarium moniliforme[8] was isolated several times during the course of these investigations, and the results presented in table 1 show that it was pathogenic under the conditions of the experiment. The isolates tested gave a rather high percentage of infection in all three host species used. Figure 4 shows cankers produced on hypocotyls of *Lupinus angustifolius*.

[6] Determined by Dr. W. C. Snyder.
[7] In a letter to the writer.
[8] Identified by Dr. D. C. Sherbakoff.

The plants were inoculated in the usual manner on October 29, 1941, and photographed on January 13, 1942. The fungus was recovered from several of the lesions. The writer doubts that this fungus is responsible for any large proportion of the root rotting of lupines observed in the field. Nevertheless, the fungus is widespread and is capable of attacking all three host species. It might, therefore,
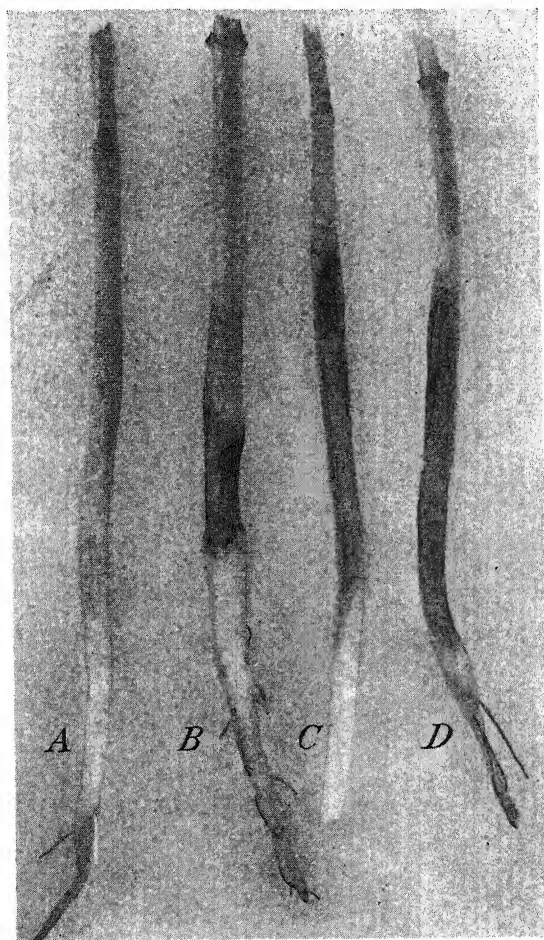
FIGURE 4.—Cankers produced by *Fusarium moniliforme* on hypocotyls of *Lupinus angustifolius*: *A*, Uninoculated control; *B* to *D*, cankers of various sizes. Photographed about 10 weeks after inoculation. × 1.

under conditions favorable for rapid development, cause considerable damage. The lesions produced by this fungus under experimental conditions were straw-colored and not very deep, but otherwise differed little from lesions caused by the other fusaria.

FUSARIUM SOLANI

A single plant of *Lupinus angustifolius*, growing in a greenhouse at Gainesville, Fla., was found to be suffering from a root rot, and

Fusarium solani [9] was isolated from it. Inoculation experiments showed this fungus to be pathogenic on all three species of the host, as denoted by the figures for the culture 1229A in table 1. Figure 5 shows four plants of *L. albus* with cankers of various sizes resulting from inoculations made just 2 weeks earlier. None of the control plants were affected. The lesions could not be distinguished from those caused by *F. oxysporum* f. *radicis-lupini*, but the rate of decay

FIGURE 5.—Plants of *Lupinus albus* (*A–D*), showing various degrees of injury, from very small canker (*A*) to destruction of entire root system (*C, D*). Inoculated with *Fusarium solani* f. *lupini* September 9, 1940; photographed September 23, 1940. × ⅖.

was slightly more rapid and the affected tissue was darker in color. There was no wilting of the foliage until the root system was badly decayed. Figure 5, *C* and *D*, illustrates the amount of dwarfing. Figure 5, *B*, shows a large lesion on the hypocotyl, yet the plant was only slightly, if at all, dwarfed.

Since the causal fungus belongs to the species *Fusarium solani* [10]

[9] See footnote 8, p. 448.
[10] Identification confirmed by Dr. W. C. Snyder

and is pathogenic to *Lupinus*, this fungus becomes *F. solani* f. *lupini* n. f. A brief description is given below. This fungus would probably be classed as a variety of *F. solani* in the Wollenweber and Reinking (*19*) system of classification.

Fusarium solani f. lupini n. f.

Macroconidia abundant, forming sporodochia and pionnotes. Spores from pionnotes on 2-percent dextrose-potato agar slants: Nonseptate, 9μ–15μ × 4μ–5.5μ; one-septate, 17μ–23μ × 5μ–5.5μ; two-septate, 25μ × 6μ; three-septate, up to 99 percent, 25μ–43μ × 5μ–6μ (average 35.9μ × 5.6μ). Sporodochia and pionnotes abundant, light brown approaching clay color, sometimes olive buff. Spores slightly curved, nearly uniform in diameter, tapering abruptly at one or both ends or often rounded at ends. Sometimes distinctly wider above or below the middle. Found causing a root rot of *Lupinus angustifolius* in the greenhouse at Gainesville, Fla.

While working on lupine root rots, the writer isolated a fungus (1240) from a hybrid pea plant (*Pisum arvense* L.) on which it was evidently causing a root rot. Since this fungus seemed to be very similar to that from lupines (1229A), its pathogenicity was tested. As indicated in table 1, it was pathogenic on both *Lupinus luteus* and *L. angustifolius*. When inoculated into Austrian Winter peas, it caused a mild type of root rot and was recovered from several of the plants. Since it is of the *Fusarium solani* type and is pathogenic to peas, it is classed as *F. solani* f. *pisi* (Jones) Snyder and Hansen.

The strain studied differs from that described by Jones (*7*) chiefly in spore size. The three-septate spores from pionnotes from 2-percent dextrose-potato agar slants, grown in a north light at room temperature (ranging from 25° to 30° C. most of the time), measured 29μ–47μ × 5μ–6.5μ (average 39.2μ × 6μ). Jones gives 27μ–37μ × 4μ–4.5μ (average 31.7μ × 4.3μ) as the size of the three-septate spores of *Fusarium martii* var. *pisi* that he studied. The writer's spore measurements fall well within the limits given for this species by Harter (*6*) so far as length is concerned. Harter made no study of the variation in spore width. He, as well as Snyder and Hansen (*14, 15*), points out that considerable variation may be expected in spores of these fungi, and this is probably the explanation for the apparent discrepancy between these two sets of spore measurements.

PYTHIUM GRAMINICOLUM

Several species of *Pythium* have been found to be associated with lupine root rots in other countries (*13*). None has been isolated from naturally infected lupine roots in the work reported here. A single experiment was conducted, however, to test the pathogenicity of 2 species of *Pythium* and 1 of *Aphanomyces*, since these cause root rots of other legumes. Both *Lupinus albus* and *L. luteus* were included in the experiment. *Pythium irregulare* Buisman and *Aphanomyces euteiches* Drechs. failed to produce any appreciable decay in the hypocotyls or fibrous roots of lupines. The inoculations were made on September 12, 1940, and 1 week later 13 out of 20 plants of *L. albus* and 1 out of 5 plants of *L. luteus* inoculated with *P. graminicolum* Subr. were diseased. The nature of the injury is illustrated in figure 6. The decayed tissue toward the ends of the lesions approached a chestnut color when moist, while that nearer the center was darker, being almost black in places. The cells contained many large strands of typical *Pythium* mycelium. The fungus was recovered from the

diseased tissue. This experiment suggests that *Lupinus albus* may be quite susceptible to *Pythium graminicolum* under conditions suitable for the latter's development. Although 1 plant of *L. luteus* developed a typical lesion, the others remained entirely healthy.
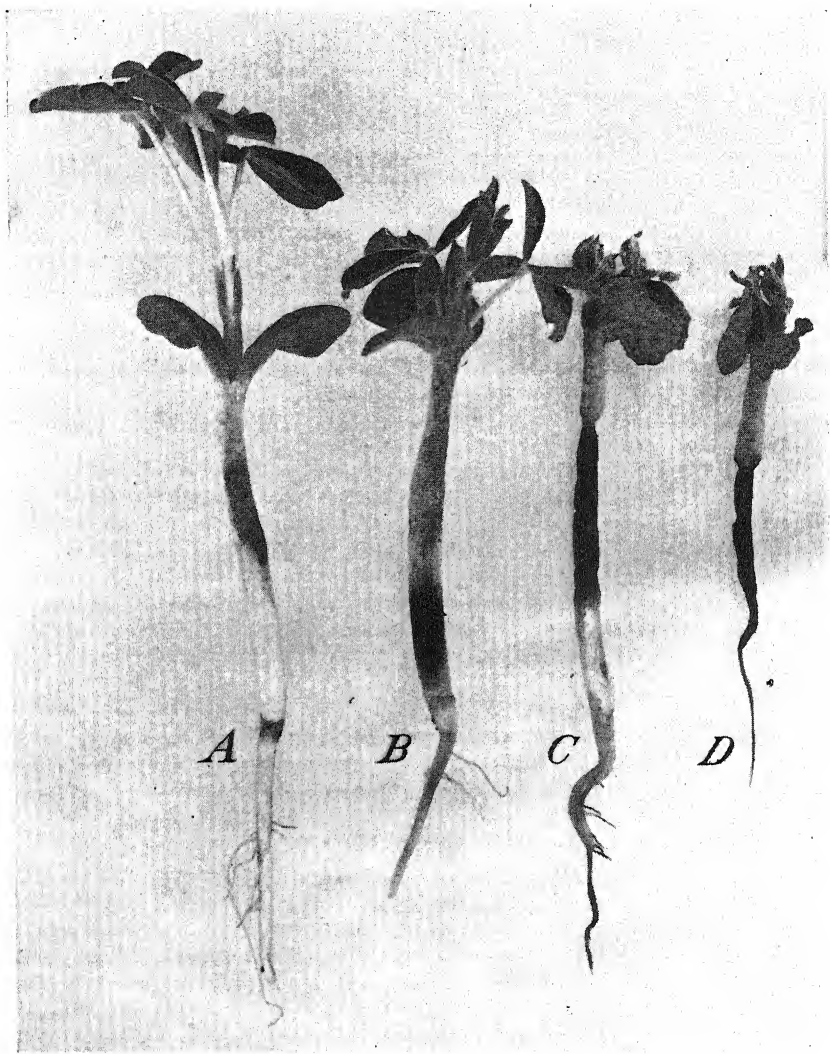
FIGURE 6.—Hypocotyl rot of *Lupinus albus*, caused by *Pythium graminicolum:* A, Plant not dwarfed as compared with controls; B to D, plants considerably smaller and about to die. Inoculated September 12, 1940; photographed 8 days later. × ⅗.

FOOT ROT

SYMPTOMS

Next to the root rots, the most common and destructive disease of lupines studied is a foot rot caused by *Sclerotium rolfsii* Sacc. This fungus produces a characteristic foot rot of lupines just as it does on

so many other plants in the South. The plants are attacked near the surface of the soil, where a lesion is formed that gradually involves the entire stem and often girdles it. The plants may at first be stunted, but later they wilt and die. Often more or less white mycelium is seen growing over the surface of the soil about the plant. Typical small, round, white sclerotia, that later turn brown, are frequently formed on the lesions or on the soil adjacent, especially in wet weather. Plants of all ages growing in the field may be killed during the spring and early summer. Often a number of neighboring plants, or consecutive plants down a row, are attacked, although isolated plants also may be killed. Sometimes a considerable number of plants in a field may succumb to the disease, but, so far as the writer's observations go, usually only isolated or small groups of plants are affected. The
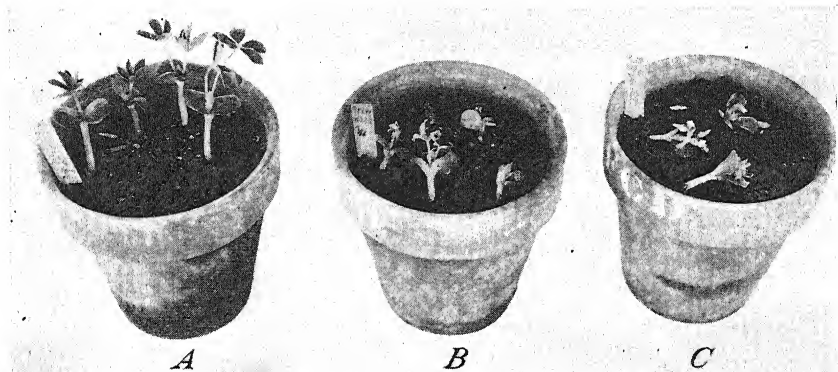
FIGURE 7.—Seedlings of *Lupinus albus: A,* Uninoculated controls; *B* and *C,* all plants decayed at the surface of the soil and fallen over, 4 days after being inoculated with *Sclerotium rolfsii.* × ²⁄₁₅.

total percentage of plants lost in a small patch at times may be high. Young seedlings may be decayed off and die within a few days, but death comes much more slowly to older plants.

INOCULATION EXPERIMENTS

The causal fungus was isolated and inoculation experiments were conducted in much the same manner as described under root rots. The chief differences were that the medium was an agar culture and sclerotia were used as the inoculum. Figure 7 shows seedlings of *Lupinus albus* decayed off near the surface of the soil 4 days after inoculation. The nature of the lesions produced by this fungus is more apparent in the plants illustrated in figure 8. This fungus penetrated the stem and severed it rather quickly. The fungus works up and down the stem also, especially when the stem is less succulent, and involves a considerable portion of it. The seedlings illustrated in figure 8 were inoculated by placing hyphae from an agar culture directly against the healthy hypocotyl. The affected tissue was water-soaked to light brown in appearance.

The fact that fully grown plants are killed suggests that plants are susceptible at all ages, but it was not known definitely when the older plants became infected. A single experiment was conducted to gain

further information on the susceptibility of older plants. *Lupinus angustifolius* plants 1 foot tall and *L. luteus* plants 4 to 5 inches tall were
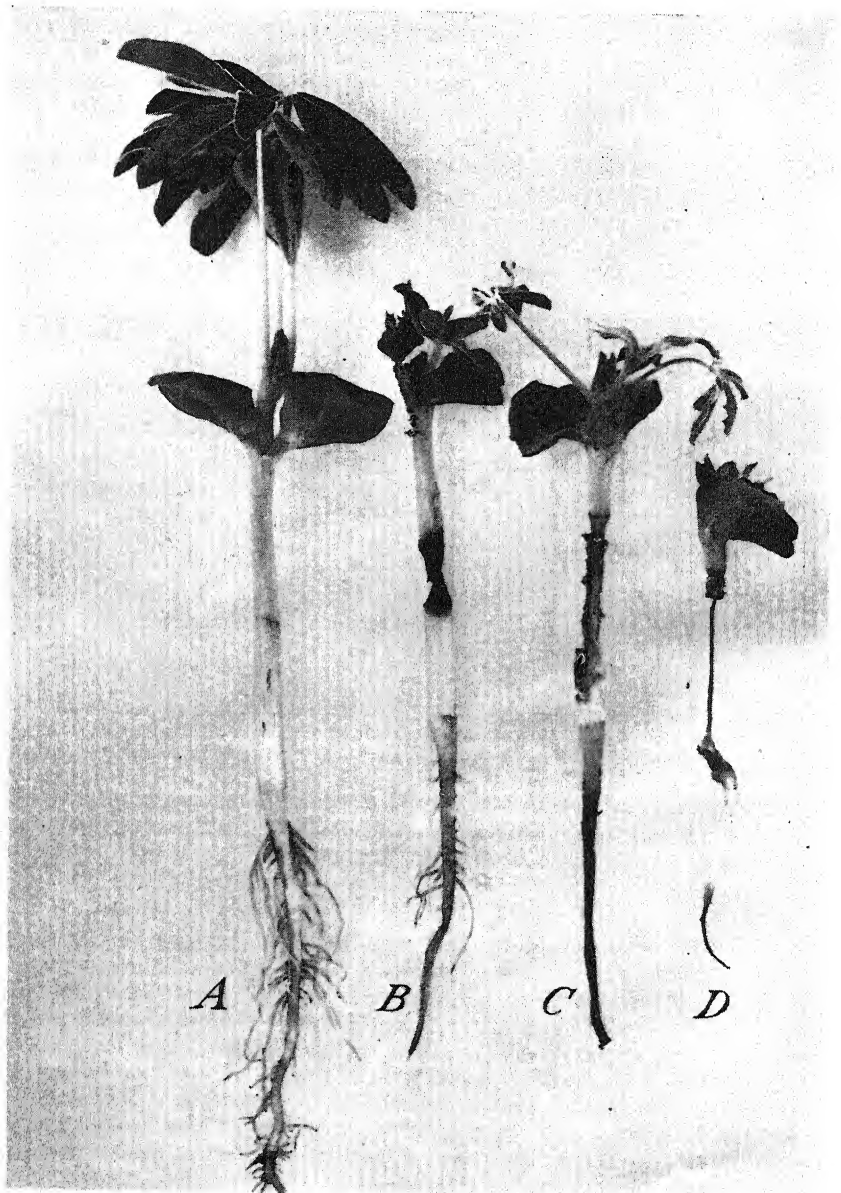
FIGURE 8.—*Lupinus albus* seedlings. *A,* Uninoculated control. *B* to *D,* Plants inoculated with *Sclerotium rolfsii,* showing injury and dwarfing: *B,* Stem almost completely severed 12 days after inoculation; *C* and *D,* the fungus has worked up and down the stem from the original point of infection. ✕ ½.

inoculated by placing sclerotia against the stems just below the surface of the soil. In order to give the fungus a better opportunity to get

started, some potato starch was placed on and about the plant and sclerotia. The inoculations were made May 3, 1939; on June 7, 3 out of 16 *L. luteus* plants inoculated had characteristic *Sclerotium rolfsii* lesions at the point of inoculation, and 8 out of 11 *L. angustifolius* plants were infected. One plant of the latter had been killed and the others had well-developed cankers. The lesions varied in color but were close to the Mars brown or the Vandyke brown of Ridgway (*12*). The diseased tissue adjacent to the healthy tissue was often liver brown in color. The lesions were sunken and had numerous strands of white aerial mycelium on the surface. There was little evidence of top symptoms until the stem was nearly girdled, and then the plant wilted and died rapidly.

DISCUSSION

The fact that a form of *Fusarium oxysporum*, belonging to the *orthoceras* group under the Wollenweber classification, was found to cause a root rot of lupines in the southeastern part of the United States is not surprising in view of the fact that Carrera and Noll (*2*) found what appears to be the same fungus pathogenic to certain species of lupines in Uruguay. They rate this fungus as possessing medium virulence. In the present work the amount of infection produced by this fungus in the different experiments varied so much that it would be difficult to rate its virulence precisely. Perhaps the fact that the amount of infection did vary would justify classing it as having medium virulence, as did Carrera and Noll, although under certain field conditions, it does appear to have a high degree of virulence. Noll (*9*) isolated several species of *Fusarium* from lupine roots, *F. oxysporum* and *F. solani* being the commonest. He states, however, that *F. orthoceras* was also pathogenic.

The question whether *Fusarium oxysporum* f. *radicis-lupini* causes a root rot or a wilt may be open to debate. The statement was made earlier in this paper (in the section on symptoms) that the disease studied was not a typical fusarium wilt, although wilting does occur as a result of the severance of the hypocotyl or the destruction of the root system. No extensive histological studies were made, but the stems of a number of plants were sectioned. The central vascular system often is somewhat discolored and some gum is present in the tissues above the region in which the cortex is seriously damaged or destroyed. Some mycelium may also be present a short distance above the decayed tissue. Carrera and Noll discussed this subject in considerable detail and concluded to call the disease caused by the species of *Fusarium* investigated by them root rot and wilt.

In general, a fusarium wilt disease is characterized by a browning of the vascular bundles, often accompanied by wilting. There may or may not be root rot present, especially in late stages of the disease; but, if present, it is commonly caused, or at least accentuated, by secondary invaders. As already pointed out, the typical symptoms of the lupine disease under discussion are a decaying of the roots and not a vascular browning. For this reason the writer prefers to consider this disease a root rot rather than a wilt. It is pertinent to note, as pointed out by Wollenweber and Reinking (*19*), that there is in Europe a typical wilt attributed to a form of *Fusarium oxysporum*, as well as root rots of lupines caused by *F. avenaceum* (Fr.) Sacc. and *F. equiseti* (Cda.) Sacc.

It is evident from these investigations that root rots of lupines in the United States are caused by several fungi. Fusaria are probably the most destructive. *Rhizoctonia* is thought by some investigators to be the most virulent pathogen attacking lupines. This may be true of plants in the seedling stage, but the evidence presented in this paper does not justify such a conclusion for older plants. It is true, however, that strains of *Rhizoctonia* differ considerably in pathogenicity (*8*), and the strain used in these investigations may not have been so virulent as some.

Schultz (*13*) points out that many species of *Pythium* cause rotting of lupine roots in Germany. Of the species tested in the present investigations, only *P. graminicolum* proved to be pathogenic. No *Pythium* was isolated from naturally infected plants, but too few isolations were made to justify the conclusion that *Pythium* does not contribute to the destruction of lupine seedlings in this country. Some seedsmen have reported that it is necessary to sow lupine seeds much thicker than would otherwise be necessary, because of the high mortality of the seedlings. The writer has made no specific investigation into the cause of the death of these seedlings, but it is assumed that *Rhizoctonia*, the fusaria, and possibly some species of *Pythium* may be the causal agents.

Although the losses caused by these diseases have been serious in local areas, largely in experimental plots, no general complaint has been received from commercial growers, a fact which indicates that losses have not been large or that their cause has not been recognized. Since there appear to be so many potential causal agents of root rots, and since all are soil-borne and some live on other hosts, finding resistant strains or species of the host is probably the only hopeful control measure.

SUMMARY

Root rots of three introduced species of lupines (*Lupinus angustifolius*, *L. luteus*, and *L. albus*), caused by several species of *Fusarium*, by *Rhizoctonia solani*, and by *Pythium graminicolum*, and a foot rot caused by *Sclerotium rolfsii* are described and illustrated.

Fusarium oxysporum f. *radicis-lupini* n. f., the pathogen most frequently isolated, is believed to be the common cause of the root rotting of lupines found in the southeastern part of the United States. *F. solani* f. *pisi*, *F. solani* f. *lupini* n. f., and *F. moniliforme* also are shown to be pathogenic under the conditions of these experiments.

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